UNIVERSIDADE DE LISBOA

Faculdade de Medicina de Lisboa

Reflex Syncope: an integrative physiological approach

Sérgio Miguel Matoso Laranjo

Supervisor: Prof. Doutora Maria Isabel de Sousa Rocha
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Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, especialidade de Fisiologia

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Any views expressed in the dissertation are those of the author.

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Lisbon. The work is original, except where indicated by particular reference in the text, and no part of the dissertation has been submitted for any other academic award.

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A note to the readers,

This thesis consists of several chapters and some complementary sections. Chapters 1 and 2 contain an organised up-to-date review of the literature, which will introduce the reader to the field and lead him/her naturally to the objectives, working hypotheses, work methodologies, results and their specific discussion (which are shown in chapters 4 to 6). A final Discussion on Chapter 7 establishes the novelty and importance of our contribution to this field of knowledge.

The present thesis has two appendices: Appendix I, consisting of information on autonomic function and its organisation, which is relevant to the thesis, but not necessary to text understanding; Appendix II, with supplementary data, tables and figures, complementing the data discussed throughout the chapters mentioned above. The references are collected at the end of the thesis. Complementing this organisation and presented in the first pages are the acknowledgements, authorship, index of figures and tables, list of abbreviations and the summary of the thesis.

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AUTHORSHIP

The results presented in this dissertation are or will be supporting the following manuscripts:

1. **Laranjo S**, Oliveira M, Rocha I. Integration of autonomic dysfunction and baroreceptor reflex behaviour to stratify patients with recurrent syncope. Submitted to Heart Rhythm Journal

2. **Laranjo S**, Geraldes V, Oliveira M, Rocha I. Insights into the background of autonomic medicine. Rev Port Cardiol. 2017 Oct;36(10):757-771. DOI: 10.1016/j.repc.2017.01.007.
3. **Laranjo S**, Tavares C, Oliveira M, Trigo C, Pinto F, Rocha I. An insight into the autonomic and haemodynamic mechanisms underlying reflex syncope in children and adolescents: a multiparametric analysis. Cardiol Young. 2015 Apr;25(4):647-54. DOI: 10.1017/S1047951114000511.
4. **Laranjo S**, Tavares C, Oliveira M, Rocha I. Autonomic modulation in a patient with syncope and paroxysmal atrial-fibrillation. Auton Neurosci. 2014 Jul;183:116-9. doi: 10.1016/j.autneu.2014.03.001.
5. **Laranjo S**, Martins Oliveira M, Tavares C, Geraldes V, Santos S, Oliveira E, Ferreira R, Rocha I. [Tilt training increases vasoconstrictor reserve in patients with neurocardiogenic syncope]. Rev Port Cardiol. 2012 Jul-Aug;31(7-8):469-76. DOI: 10.1016/j.repc.2012.05.004.
6. Tavares, C. Carneiro, R.M. **Laranjo, S**. Rocha, I. Computational tools for assessing cardiovascular variability IEEE/EMBS/ENBENG.2011.60260-82: 1-6.
7. Ducla-Soares JL, Santos-Bento M, **Laranjo S**, Andrade A, Ducla-Soares E, Boto JP, Silva-Carvalho L, Rocha I. Wavelet analysis of autonomic outflow of normal subjects on head-up tilt, cold pressor test, Valsalva manoeuvre and deep breathing. Exp Physiol. 2007 Jul;92(4):677-86.

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2. **Prémio Jorge Quininha Prize 2012(b)**: "Tilt training increases vasoconstrictor reserve in patients with neurocardiogenic syncope". **Laranjo S**, Martins Oliveira M, Tavares C, Geraldes V, Santos S, Oliveira E, Ferreira R, Rocha I. Lisboa, 2012.

3. **Prémio jovem investigador – investigação clínica do XXXV Congresso Português de Cardiologia 2014** - "The predictive value of a multivariate model of autonomic and baroreflex function applied to patients with reflex syncope". **Sérgio Matoso Laranjo**, Cristiano Tavares, Mário Martins Oliveira, Isabel Rocha. XXXV Congresso Português de Cardiologia. Albufeira, Abril de 2014.

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LIST OF ABBREVIATIONS

AF	atrial fibrillation
ANF	autonomic nervous failure
ANS	autonomic nervous system
AUC	area under the curve
BEI	baroreflex effectiveness index
BP	blood pressure
BPM	beats per minute
BRS	baroreflex sensitivity
CO	cardiac output
CPT	cold pressure test
CTR -	control
CWT	continuous wavelet transform
DB	deep breathing test
Db12	Daubechies 12
dBP	diastolic blood pressure
DWT	discrete wavelet transform
ECG	electrocardiogram
ED	emergency department
EMD	empirical mode decomposition
FFT	Fast Fourier transform
GDPR	general data protection regulation
HF	high frequencies band
HF_{RRI}	high frequencies band derived from the RRI
HHT	Hilbert Huang Transform
HR	heart rate
HRV	heart rate variability
HT	Hilbert Transform
HUT	head-up tilt table testing
IMF	intrinsic mode functions

ISQL	"Impact of Syncope on Quality of Life" scale
LF	low frequencies band
LF_{SBP}	low frequencies band derived from the sBP
LOC	loss of consciousness
mHHT	modified HHT
MSNA	Muscle Sympathetic Nerve Activity
NTS	Nucleus of the solitary tract
OH	orthostatic hypotension
QoL	Quality of life
ROC	Receiver operating characteristic curve
RRI	R-R interval
sBP	systolic blood pressure
SFS	syncope free survival
Sps	samples per second
SqA	square of the detail’s amplitude
STFT	short-time Fourier transform
SV	stroke volume
TLOC	transient loss of consciousness
TPR	total peripheral resistance
VASIS	VAsovagal Syncope International Study
VLF	very-low frequencies band
VM	Valsalva manoeuvre
VVS	vasovagal syncope

ABSTRACT

Syncope, the most common form of transient loss of consciousness, accounts for up to 5% of emergency room visits and up to 3% of hospital admissions. It is a frequent medical problem with multiple triggers, potentially dangerous, incapacitating, and challenging to diagnose. Therefore, a detailed clinical history is needed first to establish the nature of the loss of consciousness. However, after diagnosis, the therapeutic measures available are still very poor. Although the exact pathophysiology of vasovagal syncope remains to be clarified, some underlying mechanisms have been unveiled, dependent not only on the cause of syncope but also on age and various other factors that affect clinical presentation. Ultimately, syncope depends on a failure of the circulation to perfuse the brain, so any factor affecting blood circulation may determine syncope occurrence.

Thus, the purpose of the present study is to understand the impact of the hemodynamic and autonomic functions on reflex syncope mechanisms to improve patients diagnose, prognosis and general quality of life. Bearing that in mind, we designed and implemented new mathematical and computational tools for autonomic and hemodynamic evaluation, in order to deepen the understanding of their involvement in reflex syncope mechanisms. Furthermore, by refining the diagnostic accuracy, sensitivity and specificity of the head-up tilt-table test, we established a predictive tool for the impending syncopal episode. This allowed us to establish effective and personalised treatment alternatives to patient's refractory to conventional options, contributing to their increase in the quality of life and a reduction of health care and associated costs.

In accordance, in a truly multidisciplinary study involving reflex syncope patients, we were able to show an elemental functional asynchrony of hemodynamic and autonomic reflex responses, expressed through a temporal mismatch between cardiac output and total peripheral resistance adaptations, a deferred baroreflex response and an unbalanced, but incremental, autonomic tone, all contributing to autonomic dysfunction, translated into a decreased sympathetic reserve. Through the design,

testing and implementation of a computational platform and the associated software - FisioSinal platform -, we developed novel and dynamic ways of autonomic and hemodynamic evaluation, whose data lead to the development of predictive algorithms for syncope patients'risk stratification. Furthermore, through the application of these tools, we showed the effectiveness of a non-invasive, non-disruptive and integrated treatment, focusing on neuromodulation of the autonomic and cardiovascular variables involved in the syncope mechanisms, leading to a substantial increase of quality of life and the abolishment of syncopal events in a vast majority of the enrolled patients.

In conclusion, our work contributed to fill the gap between the best available scientific information and its application in the clinical practice by tackling the three pillars of translational medicine: bench-side, bedside and community.

Keywords: syncope, autonomic nervous system, baroreflex, heart rate variability, tilt training, cardio-neuromodulation

RESUMO

Síncope, a forma mais comum de perda temporária de consciência é responsável por até 5% das idas aos serviços de emergência e até 3% dos internamentos hospitalares. É um problema médico frequente, com múltiplos gatilhos, incapacitante, potencialmente perigoso e desafiante em termos diagnósticos e terapêuticos. Assim, é necessária uma anamnese detalhada para primeiro estabelecer a natureza da perda de consciência, mas, após o diagnóstico, as medidas terapêuticas existentes são pouco eficazes. Embora a fisiopatologia da síncope vasovagal ainda não tenha sido completamente esclarecida, alguns mecanismos subjacentes foram já desvendados. Em última análise, a síncope depende de uma falha transitória na perfusão cerebral pelo que qualquer factor que afecte a circulação sanguínea cerebral pode determinar a ocorrência de síncope.

Assim, o objectivo do presente estudo é caracterizar o impacto hemodinâmico e autonómico nos mecanismos subjacentes à síncope reflexa, para melhorar o diagnóstico, o prognóstico e a qualidade de vida dos doentes e dos seus cuidadores. Para isso, desenhámos e implementámos novas ferramentas matemáticas e computacionais que permitem uma avaliação autonómica e hemodinâmica integrada, de forma a aprofundar a compreensão do seu envolvimento nos mecanismos de síncope reflexa. Além disso, refinando a precisão do diagnóstico, a sensibilidade e a especificidade do teste de mesa de inclinação ("*tilt test*"), estabelecemos uma ferramenta preditiva do episódio iminente de síncope. Isso permitiu-nos estabelecer alternativas de tratamento eficazes e personalizadas para os doentes refractários às opções convencionais, sob a forma de um programa de treino de ortostatismo ("*tilt training*"), contribuindo para o aumento da sua qualidade de vida e para a redução dos custos directos e indirectos da sua assistência médica.

Assim, num estudo verdadeiramente multidisciplinar envolvendo doentes com síncope reflexa refractária à terapêutica, conseguimos demonstrar uma assincronia funcional das respostas reflexas autonómicas e hemodinâmicas, expressas por um desajuste temporal entre o débito cardíaco e as adaptações de resistência total periférica, uma resposta baroreflexa atrasada e um desequilíbrio incremental do tónus autonómico

que, em conjunto, poderão resultar de uma disfunção do sistema nervoso autónomo que se traduz por uma reserva simpática diminuída. Igualmente, desenhámos, testámos e implementámos uma plataforma computacional e respectivo *software* associado - a plataforma FisioSinal –incluindo novas formas, mais dinâmicas, de avaliação integrada autonómica e hemodinâmica, que levaram ao desenvolvimento de algoritmos preditivos para a estratificação de doentes com síncope. Além disso, na aplicação dessas ferramentas, comprovámos a eficácia de um tratamento não invasivo, não disruptivo e integrado, focado na neuromodulação das variáveis autonómicas e cardiovasculares envolvidas nos mecanismos de síncope. Esta terapêutica complementar levou a um aumento substancial da qualidade de vida dos doentes e à abolição dos eventos sincopais na grande maioria dos doentes envolvidos.

Em conclusão, o nosso trabalho contribuiu para preencher a lacuna entre a melhor informação científica disponível e sua aplicação na prática clínica, sustentando-se nos três pilares da medicina translacional: investigação básica, clínica e comunidade.

Palavras-chave: Síncope, sistema nervoso autónomo, baroreflexo, variabilidade da frequência cardíaca, treino de ortostatismo, neuromodulação

CHAPTER 1

CHAPTER 1

STATE OF THE ART ON REFLEX SYNCOPE

1.1 General Overview of Syncope

Sudden disturbances of consciousness are one of the most critical problems in clinical practice since they can be a sign of various cerebral and somatic pathologies. Episodes of transient loss of consciousness (TLOC) may be due to a large variety of causes, including syncope, epileptic seizures, autonomic failure, cardiac arrhythmias and functional disorders.

Syncope, the most common form of TLOC, accounts for up to 5% of emergency room visits and up to 3% of hospital admissions (Day, Cook, Funkenstein, & Goldman, 1982; Silverstein, Singer, Mulley, Thibault, & Barnett, 1982; Morichetti & Astorino, 1998; Brignole et al., 2001, 2018; Moya et al., 2009). It is a frequent medical problem, potentially dangerous, incapacitating and challenging to diagnose.

The literature on syncope and associated conditions can be confusing because of the lack of consistency (Brignole et al., 2001, 2018; Moya et al., 2009). There have been many clinical, scholarly and colloquial definitions proposed for syncope. Different terms that may refer to syncope are used in everyday English, including, *blackout, collapse, faint, fit, spell, dizzy, funny turns, breath-holding spells, drop attacks and giddiness* (Brignole & Benditt, 2011b). The term syncope has its origins in the ancient Greek συγκοπή (sunkopḗ), meaning cessation, interruption or sudden pause. The lack of common ground is detrimental to patient care, resulting in a high rate of incorrect diagnoses and, consequently, in inefficient and costly investigations.

The present chapter provides a common ground for TLOC. Its primary purpose is to present an overview of the field through a classification designed to aid differential diagnosis.

1.2 Definition of Transient Loss of Consciousness and Syncope

Consciousness is defined as the capacity to preserve self-awareness and to react to one's environment; unconsciousness is a situation in which this capacity is lost and the response to environmental stimuli is markedly reduced (Adams, Victor, & Ropper, 1997; Furlan, Costantino, Solbiati, & Alboni, 2015). Loss of consciousness (LOC) can last briefly and be resolved without clinical intervention, extend itself until a specific cause is treated and, then, being followed by either a complete recovery or with postictal residual neurological symptoms; and finally, LOC can also be sustained indefinitely (Furlan et al., 2015). In syncope, the duration of LOC is short, usually lasting no more than 20 seconds (Wieling et al., 2009). However, there is wide variability in the duration of episodes among different subjects.

According to the European Society of Cardiology Guidelines, syncope can be defined as a "*TLOC due to cerebral hypoperfusion, characterised by a rapid onset, short duration, and complete spontaneous recovery*" (Brignole et al., 2018). Presyncope is characterised by a feeling of discomfort with a restriction of the state of consciousness, such that the individual is aware of the loss of consciousness. Presyncope symptoms are usually not specific (dizziness, asthenia, blurred vision, nausea, difficulty in maintaining the erect position) and often overlap with those associated with the prodromal phase of syncope.

1.3. Clinical Classification of Syncope

The classification of syncope is mainly based on the underlying mechanisms, which ultimately lead to global hypoperfusion and TLOC (Puppala, Dickinson, & Benditt, 2014). These mechanisms are at the core of the pathophysiological classification of syncope proposed by the European Society of Cardiology, emphasising groups of disorders with common pathophysiology, presentation, and risk (Moya et al., 2009; Brignole et al.,

2018). This classification contains three main categories, including the reflex (neurally-mediated) syncope, the cardiac syncope, and the syncope secondary to orthostatic hypotension (Figure 1). Each category is further divided into several types, based on the pathophysiological mechanism. According to this classification, reflex syncope is divided into four main categories: vasovagal, situational, carotid sinus syndrome, and non-classical forms.

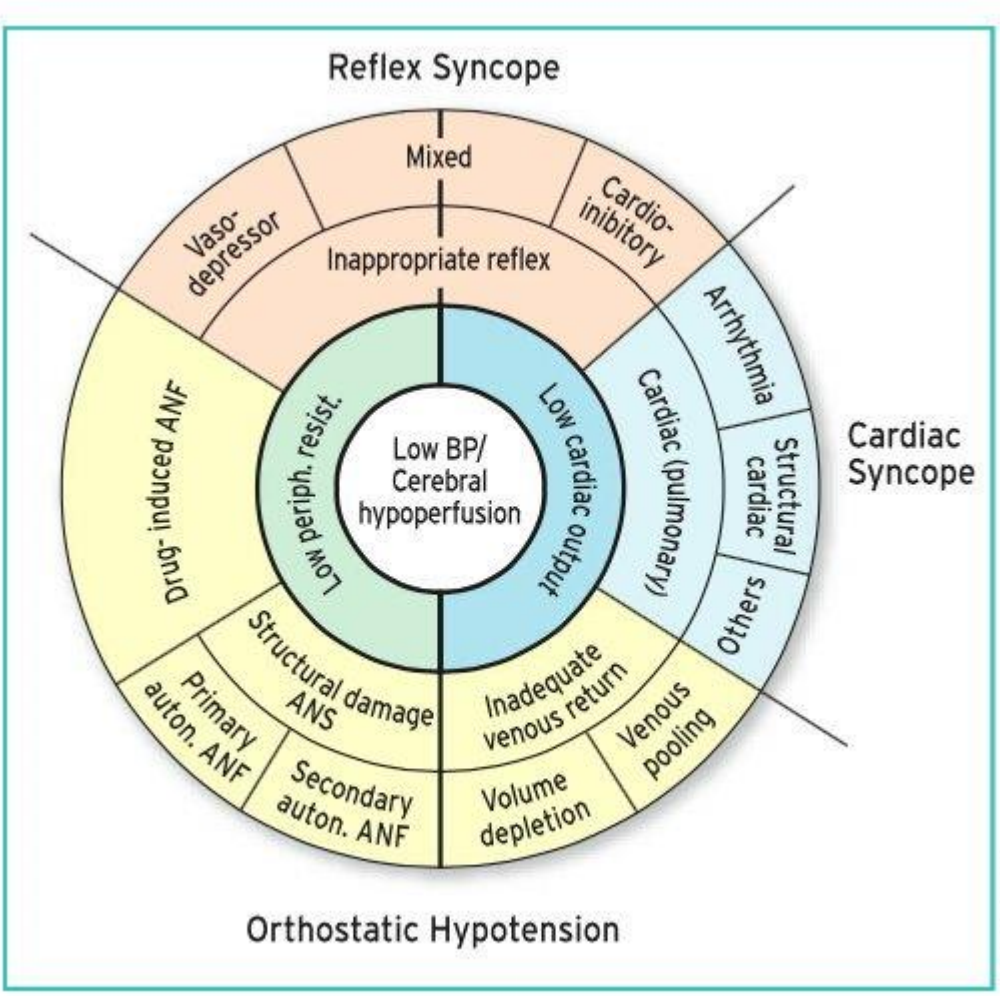


Figure 1. The pathophysiological basis for syncope classification.
European Society of Cardiology pathophysiological classification of syncope (From (Moya et al., 2009)*, with the permission of Eur Heart J). BP - blood pressure, ANS - autonomic nervous system, ANF - autonomic nervous failure, OH - orthostatic hypotension; low periph. Resist - low peripheral resistance*

Within the present work, *we will mainly focus on the orthostatic form of vasovagal syncope*, which is the most commonly observed in the clinical practice (Brignole et al., 2018). Vasovagal syncope may further be divided according to the primary

pathophysiological mechanism into the *vasodepressor syncope*, where a decrease in blood pressure prevails, and the *cardioinhibitory* type, where spontaneous bradyarrhythmias, up to asystole, dominate. The *mixed* form of syncope is present when both mechanisms are involved in the pathogenesis of syncope.

1.4. Epidemiology of Vasovagal Syncope

Syncope in general, and particularly vasovagal syncope, is a common clinical condition. Its real incidence is impossible to predict owing to variability in concepts and definitions, fluctuations in population prevalence and under-reporting in the general population (Solbiati & Sheldon, 2015) as, due to the transitory nature of the episode, most patients are asymptomatic on arrival to emergency departments. Thus, it is understandable that only a small percentage of patients with syncope search for medical advice and care (Soteriades et al., 2002; Ganzeboom, Colman, Reitsma, Shen, & Wieling, 2003; Ganzeboom et al., 2006; Serletis, Rose, Sheldon, & Sheldon, 2006).

Precise epidemiological assessment of syncope is difficult (Brignole & Benditt, 2011a). Early community-based studies provided insight into the cumulative incidence of presumed syncope. However, the estimates were, at times, quite varied. For instance, in the first report from the Framingham study, it was found that during the first 26 years of follow-up (from 1952 to 1978), only 3.2% of adults (3% of the men and 3.5% of the women) had at least one syncope episode (Savage, Corwin, McGee, Kannel, & Wolf, 1985). By contrast, the latest report derived from the study provided a substantially higher cumulative incidence of syncope (Soteriades et al., 2002). Indeed, among 7814 Framingham participants, 822 (~10%) reported having experienced, at least, one syncope attack. On this same line is another large retrospective community-based study of more than 1900 adults, reporting that 364 (~19%) subjects had experienced a syncopal episode (Chen, Shen, Mahoney, Jacobsen, & Rodeheffer, 2006a).

Apart from the above-mentioned studies, some other reports have examined the cumulative incidence of syncope in more highly selected populations (Brignole & Benditt, 2011a). Studies involving subjects from the United States Air Force personnel revealed that 27% had experienced at least one syncopal attack during their lifetime (Lamb, Green, Combs, Cheeseman, & Hammond, 1960; Kiran et al., 2017) and, in a cross-sectional survey, by questionnaire, of almost 400 Dutch medical students, it was shown that 39% of the inquired subjects had experienced at least one episode of syncope (Ganzeboom et al., 2003). Female students reported a higher prevalence of syncope than males (47% vs 24%). Median age at which students experienced their first syncope was 15 years old, for both women and men. In a Portuguese report of 2,047 young adults (aged 18–40 years), about 30 % reported a previous episode of transient loss of consciousness (Providência, Silva, Mota, Nascimento, & Leitão-Marques, 2011). Similarly to the previous studies, syncope was more prevalent in female subjects (OR 2.4, 95 % CI 2.0–2.9). Altogether, these studies suggest a cumulative syncope incidence of approximately 40% (Parry & Tan, 2010; Angus, 2016). In other words, it may be estimated that almost a third of the world's population will suffer at least one episode of vasovagal syncope in their lifetime (Kenny, Bhangu, & King-Kallimanis, 2013).

The incidence of first-time syncope by age is bimodal (Figure 2). Its prevalence is very high in patients aged 10-30 years, being very common in children, not so common in adults with an average age of 40 years old, and peaking again in patients aged >65 years (Lombroso & Lerman, 1967; Soteriades et al., 2002; Ganzeboom et al., 2003; Brignole et al., 2018), which may be related not only with ageing, but also to the prescription of vasoactive drugs and the prevalence of cardiac arrhythmias in this population (Parry & Tan, 2010). Kanjwal & Calkins reported 126 syncopal episodes for every 100,000 children (Kanjwal & Calkins, 2015), which has been epidemiologically confirmed by others (Lewis & Dhala, 1999; Kapoor, 2000). Moreover, one in every two-thousand children is admitted to emergency departments due to syncope (Kanjwal & Calkins, 2015). Indeed, in most patients, reflex syncope began in adolescence and at a young age (Kenny et al., 2013). The first episode usually occurs between the ages of 10 and 30, with a peak in females around 15 years (47% of women and 31% of men) (Gillette & Garson, 1992; Driscoll, Jacobsen, Porter, & Wollan, 1997; Ganzeboom et al., 2003; Massin et al., 2004;

Wieling, Ganzeboom, & Saul, 2004b; Serletis et al., 2006; Moya et al., 2009; Brignole et al., 2018; Müller & Paul, 2018). On the contrary, the frequency of epileptic seizures in the same age group is much lower (less than 1%), and syncope associated with arrhythmias is even less frequent (Colman et al., 2004).

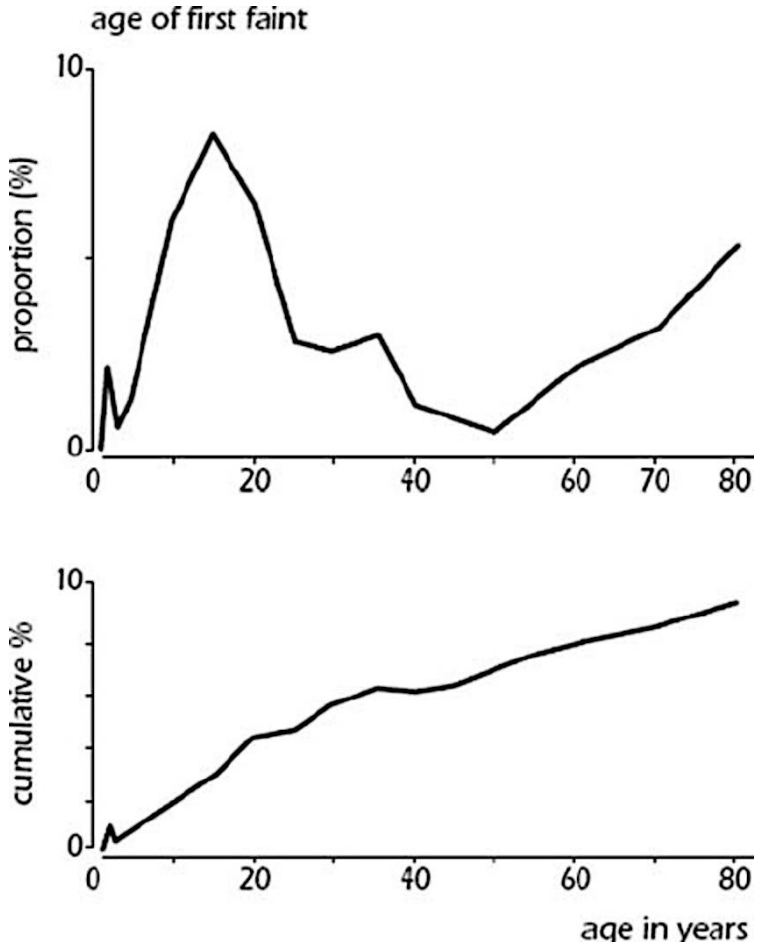


Figure 2. Schematic presentation of the distribution of age and cumulative incidence of the first episode of syncope in the general population from subjects up to 80 years.
The data from subjects 5–60 years come from a study by (Ganzeboom et al., 2003). *The data from subjects younger than 5 years are based on those of* (Lombroso & Lerman, 1967) *and those from subjects aged 60–80 years on the study by* (Soteriades et al., 2002)*(From* (Moya et al., 2009)*, with the permission of Eur Heart J)*

About thirty per cent of persons who have had a syncope attack will experience a recurrence during the three years that follow, with most recurrences occurring within the first two years (Moya et al., 2009; da Silva, 2014). Key predictors of a higher tendency to syncope recurrences include age <45 years at initial presentation, female sex and a history (generally over many years) of prior syncope recurrence. In particular, patients with multiple syncope events in the preceding year and more than six-lifetime syncope

attacks and a positive tilt-table test have a high risk of syncope recurrence (>50% over two years) (Sheldon, Rose, Flanagan, Koshman, & Killam, 1996; Grimm et al., 1997; Malik, Koshman, & Sheldon, 1997; Aydin et al., 2009; Brignole et al., 2009).

1.5. Health Economics and costs associated with syncope

Syncope accounts for approximately 1% of all the emergency department (ED) visits based on data derived from various studies in Italy, France, and the United States of America (USA) (Gendelman, Linzer, Gabelman, Smoller, & Scheuer, 1983; Blanc et al., 2002; Disertori et al., 2003; Brignole, Menozzi, et al., 2006; Moya et al., 2009; Nordkamp et al., 2009; Brignole & Benditt, 2011c; Sheldon et al., 2011; Brignole et al., 2018). In the USA, for instance, this percentage translated into >1127 million ED visits in 2006, based on 'primary diagnoses' of 'syncope and collapse' recorded in the 2006 National Hospital Ambulatory Care Survey, and > 411,000 hospital admissions when these diagnoses were listed among discharge diagnoses (Brignole & Benditt, 2011c).

Until very recently, little attention has been paid to the direct and indirect costs associated with syncope. Indeed, the calculation of the total cost of diagnosing and treating syncope is complex since the indirect costs related to loss of earnings by patients and family members are difficult to assess (Brignole & Benditt, 2011c). Nevertheless, direct costs alone are substantial and, in no small measure (about 75% of them), are driven by the relatively high 'hospitalization rate' after the initial visit to the ED or clinic (Sun, Emond, & Camargo, 2004, 2005; Brignole & Benditt, 2011c), which is understandable as physicians, due to the risk of sudden death, often take a cautious approach by admitting most of these patients to the hospital (Angus, 2016). In the USA, a conservative estimate obtained from the US Medicare database revealed the annual cost of $5.4 billion for syncope-related hospitalizations (Sun et al., 2005). In the UK, 'syncope and collapse' are among the six major causes of ED admission, with an estimated cost of up to £70 million annually for syncope care (Kenny, O'Shea, & Walker, 2002; Brignole & Benditt, 2011c). In Spain, the direct costs of syncope care in a large tertiary care facility in Seville were estimated in up to €11200/patient (Barón-Esquivias et al., 2006), whereas in Portugal, the average cost of a syncope event was estimated to

be between €1760 and €2800/patient, based on Portuguese cost data and landmark papers (Providência et al., 2014).

Assessment of syncope has been proved to be a very costly medical endeavour and yet remains inefficient. Testimony of the difficulties of managing syncope is the diversity of diagnostic procedures performed and the fact that these patients are usually followed by a multitude of medical specialities such as cardiology, neurology, paediatrics, geriatrics, internal medicine, otolaryngologist and orthopaedics. When admitted to the hospital, these patients often undergo expensive and repeated investigations, many of which are unnecessary and with low diagnostic yield, not providing a definitive diagnosis (Kapoor, Peterson, Wieand, & Karpf, 1987; Shiyovich, Munchak, Zelingher, Grosbard, & Katz, 2008; Brignole & Benditt, 2011c; Benditt & Adkisson, 2013). In the absence of a reasonably priced and straightforward standard test, which could make it possible to establish the exact cause of syncope, the extensive use of numerous ineffective diagnostic methods leads to an increase in the costs of the examination. This calls for the need for urgent improvements in diagnostic and treatment algorithms so that patients' quality-of-life may be improved. Recent European and American recommendations (Shen et al., 2017a; Brignole et al., 2018) regarding the assessment of syncope in the ED and the development of specialised multidisciplinary syncope clinics may play an essential role in patient management. Syncope Units will hopefully allow the optimisation of diagnostic workflows and reduction of the syncope-associated costs (Kenny et al., 2015). Further studies will be needed to clarify the real cost-effectiveness impact of these units when compared with the standard of care (Viqar-Syed, Bradley, & Shen, 2013).

1.6. Impact on Quality of Life

The social impact of syncope is substantial. Syncope might lead to injury to the patient or others (Brignole & Benditt, 2011c). Significant morbidity, such as fractures and accidents, are reported in up to 6% of the patients, and minor injuries in up to 30% of patients (Moya et al., 2009). Apart from physical injury, recurrent syncope may be associated with moderate to severe mental and functional incapacity (Puppala,

Sakaguchi, Dickinson, & Benditt, 2013) – like the one reported in other chronic disease states, such as rheumatoid arthritis, epilepsy, and psychiatric disorders (van Dijk et al., 2007). Syncope remains the primary cause of mortality and morbidity among older patients, causing them several social, personal, and economic hazards, with trauma posing as one of the most critical consequences at this age (Solbiati & Sheldon, 2015).

1.7. Clinical Characteristics

Reflex syncope is often triggered by situations such as prolonged orthostatic position, in hot or crowded environments. Besides, many patients experience a syncopal episode during viral events, secondary to taking medication or due to mild dehydration, often associated with inadequate fluid and salt intake or, in females, occurring during the menstrual cycle. Other individuals with situational syncope usually have stereotyped triggers that determine recurrent episodes (Table 1).

Table 1. Triggers associated with reflex situational syncope.

Airway Stimulation	**Urinary Voiding**
Apnea	**Migraine**
Carotid sinus compression	**Oculovagal reflex**
Cold drinks	**Post-prandial period**
Cough	**During medical procedures**
Defecation	**Shaving**
Apnoea diving	**Sneezing**
After intense physical exercise	**Stretches**
Glossopharyngeal reflex stimulation	**Deglutition**
Hair combing	**Playing wind instruments**
Altitude	**Valsalva manoeuvre**
Hot shower	**Vomiting**
Hyperventilation	**Weight loss**
Immunisations	

A reflex syncope event usually consists of three phases (Wieling et al., 2009; Wieling, van Dijk, et al., 2014): the prodromal phase, the loss of consciousness, and the recovery period. The former can last from seconds to minutes and is often remembered by the patient, although it is short-lived. Common symptoms include dizziness, confusion, nausea, abdominal pain, hot or cold sensation, diaphoresis, hearing or vision changes, headache and anticipation of the loss of consciousness. The next phase, characterised by loss of consciousness, lasts from a few seconds to a few minutes (usually 5-20 seconds) and is not recalled by the patients, although some have the feeling of being "turned off," able to hear the voices of those who are present but unable to respond. During this phase, observers describe the patient as pale, livid, with cold skin and profuse sweating, pupillary dilatation (occasional), and incontinence (rare). The recovery phase lasts from 5 to 30 minutes (and sometimes up to a few hours) and is characterized by fatigue, vertigo, weakness, headache, and nausea, with subsequent return to normal condition.

1.8. Prognosis

The prognosis of a patient with syncope depends on the underlying cause, since the transient loss of consciousness may be a symptom of both the autonomic nervous system and cardiac, neurological, metabolic and psychiatric disorders, with a poorer prognosis for cardiac syncope. The prognosis is particularly severe and unfortunate, whenever it involves syncope secondary to cardiac arrhythmias or structural diseases, reaching a mortality equivalent to 18-30% (Bass et al., 1988; Pires et al., 2000; Soteriades et al., 2002).

Most episodes of syncope in the general population are included in the reflex syncope group, characterised by benign prognoses; however, some potentially life-threatening cardiac conditions may have as initial manifestation a syncopal episode. Also, the patient may be at risk of sudden falls due to loss of consciousness and postural tone.

Therefore, to improve the diagnostic approach and thus the prognosis, it is fundamental to follow a specific and rigorous protocol that allows the identification of the individual at risk of mortality (Serrano et al., 2010).

1.9. Clinical assessment

The clinical features characterizing TLOC, are usually derived from history taking from patients and eyewitnesses (Wieling, van Dijk, et al., 2014). When a patient first presents with possible TLOC, history taking should first establish whether TLOC actually occurred, which often allows a distinction between major TLOC groups.

The initial evaluation of a patient with a syncopal episode should provide the physician with the necessary elements to confirm the diagnosis, to identify the aetiology and to be able to design the future diagnostic plan (Brignole et al., 2018). A diagnostic protocol that includes family and personal anamnesis, taken from patients and eyewitnesses, a complete physical examination with the evaluation of vital parameters, the measurement of blood pressure in clinostatism / orthostatism, and an ECG has been suggested to identify cardiac syncope with a sensitivity equivalent to 96% (Ritter et al., 2000; Elesber, Decker, Smars, Hodge, & Shen, 2005). The timing of the ECG or other examinations and the programming of the diagnostic plan depend on the risk stratification that is performed in this first phase. The aim is to identify events, symptoms or alarm signals suggestive of a potential cardiac aetiology of syncope (Table 2).

Table 2. Events, symptoms or alarm signals suggestive of a potential cardiac aetiology of syncope

Family History
Sudden death in a family member under 40 years old
Arrhythmia or hereditary heart disease (Long QT, cardiomyopathy)
Acute myocardial infarction in a family member under 35 years old
Personal Background
Congenital or acquired cardiopathy
Documented cardiac arrhythmia
Suspected heart disease (intolerance to physical exertion, recent asthenia)
Anamnesis
Syncope preceded by palpitations or chest pain
Syncope triggered during physical exercise or emotional stress
Syncope during swimming
Syncope in the supine position (lying)
Syncope without prodromes
Syncope after intense/irritating noise
An event requiring cardiopulmonary resuscitation manoeuvres
Event with neurological sequelae
Physical Examination
Irregular rhythm
Pathological heart tones and murmurs
Pericardial friction
Absent pulses
Altered ECG

The clinical assessment plays a central role in the diagnostic evaluation. It should include family history, anamnesis, drugs and/or substances eventually ingested, environmental factors, how the syncopal episode began, investigation of the triggering factors, prodromal phase symptoms, characterization of the episode of loss of consciousness and of the post-critical period, with a description of the event and its duration,

associated signs and / or symptoms (Table 3). An anamnesis correctly and systematically performed, can be diagnostic in up to 45% of syncope cases or, at least, it would be a remarkable help to build the best evaluation strategy (Keissar, Maestri, Pinna, La Rovere, & Gilad, 2010; Parry & Tan, 2010; Benditt & Adkisson, 2013; Wieling, van Dijk, et al., 2014; Angus, 2016).

Table 3. Important anamnestic data

Assessment of circumstances immediately before syncope
Position (lying down, sitting or standing)
Activities (at rest, after changing posture, during or after exercise, during or immediately after urination, defecation, coughing or swallowing)
Predisposing factors (e.g., hot and crowded places, prolonged orthostatic position, postprandial period) and triggering factors (fear, intense pain, neck movements)
Evaluation of the prodromes
Nausea, vomiting, abdominal pain, chills, sweating, aura, neck or shoulder pain, blurred vision
Description of the episode (witness data)
The fall (sudden drop or 'slipped' to the floor by bending the knees), skin colour (pallor, cyanosis, flushing), duration of loss of consciousness, presence of movements (tonic, clonic, tonic-clonic or minimal myoclonus, automatism, symmetry) and their duration, onset of the movements and their relation to the fall (before, during, after), tongue biting
Symptoms on recovery
Nausea, vomiting, sweating, chills, confusion, muscle aches, skin colour, lesions, chest pain, palpitations, sphincter incontinence
Relevant personal history
Family history of sudden death, congenital heart disease, or history of arrhythmic syncope
Personal history of heart disease
History of neurological disease (epilepsy, narcolepsy)
Metabolic disorders (i.e., diabetes)
Drugs (antihypertensive, antianginal, antidepressants, antiarrhythmics, diuretics or drugs that may prolong QT interval)
(In case of recurrent syncope) Information on recurrence, as well as the time elapsed since the first syncope and the number of episodes

ECG represents a fundamental instrumental examination in the initial evaluation of the patient with syncope, and several authors confirmed its usefulness (Shen et al., 2017a;

Brignole et al., 2018). As an example, in a retrospective study of 234 patients, ECG changes were found in 25 (10.7%) of the evaluated subjects (Hegazy, Lotfy, Ammar, & Fattouh, 2008). ECG should be performed routinely in patients with syncope and reported by a cardiologist as, in addition of its usefulness in identifying arrhythmic conditions such as Long QT, Short QT and Brugada syndromes, it may raise suspicion of underlying structural heart disease (Table 4 and Table 5). Moreover, an abnormality in the baseline ECG is a possible predictor of cardiac syncope (Massin, Malekzadeh-Milani, & Benatar, 2007; Brignole et al., 2018). Besides, some authors have shown that the interpretation of the ECG by a cardiologist can reduce the risk of non-diagnosis of a potentially fatal arrhythmic condition (Wathen, Rewers, Yetman, & Schaffer, 2005).

Table 4. Electrocardiographic abnormalities diagnostic of arrhythmic syncope

Mobitz type 2 second degree atrioventricular block (AVB) or complete AVB
Alternate right and left bundle branch block
Fast paroxysmal supraventricular tachycardia or ventricular tachycardia
Malfunction of an implantable cardiac device with asystole

Table 5. Electrocardiographic changes suggestive but non-diagnostic of arrhythmic syncope

Bifascicular block (defined as left bundle branch block or right bundle branch block associated with anterior or posterior left hemiblock)
Other intraventricular conduction abnormalities (QRS duration ≥ 0.12 sec)
Sinus bradycardia (HR dependent on age) or repetitive sinus blocks or sinus pauses > 3 sec
Ventricular pre-excitation
Long QT
Brugada type 1 pattern
Negative T waves in precordial leads, epsilon waves and late ventricular potentials suggestive of right ventricular arrhythmogenic cardiomyopathy
Q waves suggestive of myocardial infarction

Other exams such as echocardiography, head-up tilt table testing, ambulatory cardiac monitoring, and electrophysiological study, should only be performed if indicated,

according to the clinical-anamnestic data and the initial electrocardiographic evaluation, and should follow current in-use guidelines (Shen et al., 2017a; Brignole et al., 2018).

Role of Head-up Tilt-Table Testing

Head-up Tilt Table Testing (HUT), while initially developed to study physiological compensatory responses to orthostatic stress (Saal, Thijs, & Van Dijk, 2016), proved to be a useful diagnostic test for reflex syncope (Kenny, Bayliss, Ingram, & Sutton, 1986), nowadays being widely used in clinical practice with a variety of protocols, variants and extensions. HUT may provide an essential addition to history taking if the initial evaluation does not yield a definitive or highly likely diagnosis; nevertheless, it should neither replace the history taking nor be singly evaluated.

HUT aims to unleash a syncopal event, with recognition of symptoms, and to demonstrate pathophysiological correlation (Saal et al., 2016; Brignole et al., 2018). Both aspects are crucial to the diagnosis: recognition concerns to subjective sensations reported by the patient, as well as physical features, such as changes in facial colour or movements, reminded by eyewitnesses as similar to spontaneous ones. Together with the demonstration of pathophysiological measurements, a clinical-pathophysiological correlation is obtained, suggesting the cause of TLOC.

For most indications, HUT relies on prolonged orthostatic stress in the near-vertical position ('head-up tilt'). Since its introduction in the diagnostic workflow several protocols have been designed, with variations in the initial stabilisation phase, duration, tilt angle, type of support, with or without pharmacological challenges (Moya et al., 2009; Forleo et al., 2013; Sutton, 2013). The available evidence pinpoints that 20-60 minutes is the optimal time for the diagnosis of vasovagal syncope VVS (Forleo et al., 2013; Saal et al., 2016; Brignole et al., 2018). There is no consensus as to whether HUT, with or without pharmacological provocation, is to be preferred, as adding pharmacological provocation increases the test sensitivity by facilitating the syncope and, thus, the number of false-positive results (Forleo et al., 2013).

Despite the different protocols, some general standards were published in 1996 regarding the protocol itself and the HUT laboratory environment. The room where the test is performed should be noise-free, with regulated temperature and diffuse light. Patients should be fasted for at least 2 hours and remain in the supine position 15 to 20 minutes before raising the tilt table. It is advisable to monitor non-invasively and continuously the haemodynamic parameters (Brignole et al., 2018). The tilt table should be able to quickly assume the orthostatic position and resume the supine position with equal rapidity (in less than 15 seconds) when the test is completed to avoid the consequences of prolonged loss of consciousness.

The role of HUT in the diagnosis of VVS has been long debated (Sheldon, 2005). Syncope during HUT probably reflects a tendency towards hypotension in the upright position (Sutton & Brignole, 2014). This tendency is universal and does not only concern VVS, but also influences the clinical expression of some forms of cardiac syncope (Sutton & Brignole, 2014). Nevertheless, estimates of sensitivity and specificity of HUT to test for VVS differ due to various factors, the most significant being the use of pharmacological provocation, the (patho)physiological measurements and whether or not complaint and event recognition are considered (Forleo et al., 2013; Saal et al., 2016; Brignole et al., 2018; Cheshire & Goldstein, 2019)

Head-up tilt is not entirely restricted to diagnosis and may have preventive/protective characteristics. Patients may exhibit a decrease in syncope frequency after a HUT, which may be due to patients having learned to recognise impending syncope early, allowing them to take the appropriate measures (van Dijk et al., 2006; Sheldon et al., 2007; Wieling et al., 2015).

1.10 Therapeutic approaches

The therapeutic approach of syncope includes non-pharmacological behavioural and pharmacological therapeutic strategies (Brignole et al., 2018) acting on the various levels of the reflex arc, which triggers the syncopal episode (Table 6).

Table 6. Therapeutic strategies for treating patients with reflex syncope

Strategy	Patient Population
Explanation of the diagnosis, provision of reassurance, and explanation of the risk of recurrence.	In every patient
Increase in ingestion of salt and water	In every patient.
Isometric physical counterpressure manoeuvres	In patients with prodromal symptoms
Tilt training	In selected and motivated patients with recurrent episodes refractory to other measures
Drugs	No evidence of effectiveness apart from beta-blockers in patients older than 42 years
Cardiac pacing	In patients older than 40 y, with unexpected recurrent syncope in whom asystole is documented during spontaneous syncope or elicited by adenosine 5'-triphosphate.

Reproduced with permission from (Moya, 2015)

The therapeutic approach to any patient who suffers from recurrent vasovagal syncope must be individualized (Grubb, 2005). It is mainly based on two significant demands: relieving the symptoms and improving the prognosis (Moya, 2015).

In most patients, syncope occurs infrequently and only under exceptional circumstances. Some individuals, however, suffer from recurrent syncopal episodes with a quite brief, or even absent, prodromal phase, while others will not respond to the above-mentioned conservative measures. For these patients, the therapeutic options available are few, vexing and mainly palliative rather than curative. Indeed, it has been noted that the evaluation of any therapy in reflex syncope "*has been undermined by a variety of factors, including difficulty in demonstrating the efficacy of therapy under controlled conditions, unrealistic endpoints (i.e., a goal of eliminating all symptoms) and inadequate understanding of the natural history of the problem*." (Sra, 2001).

I. Non-Pharmacologic Treatments

The non-pharmacologic options involve counterpressure manoeuvres, salt and fluid intake, and orthostatic training, which may bring important results for reflex syncope patients, if combined with counselling and education of the patients and their families (Shen et al., 2017b; Brignole et al., 2018). Regarding this point, patients should, firstly, be reassured about the benignity of the episodes and instructed on the mechanisms of action of reflex syncope to prevent syncope at the onset of prodromal symptoms and to perform manoeuvres preventing its development (Vaddadi, Corcoran, & Esler, 2010). These include avoidance of hot or crowded environments or of dehydration leading to hypovolemia, together with avoidance of other known predisposing factors, like sudden postural changes. Drugs that could potentially enhance a person's predisposition to syncope should be identified and, if possible, discontinued. Patients should also be advised to lie down at the onset of any premonitory symptoms and not remain standing or sitting. Measures that increase venous return, provided they are not contraindicated, such as volume expansion with water intake (3L/day), the liberalisation of salt consumption (6g/day), can be useful, although the evidence of their effectiveness is limited (El-Sayed & Hainsworth, 1996; Schroeder et al., 2002; Wieling & Hainsworth, 2002; Lu et al., 2003; Claydon & Hainsworth, 2004; Claydon, Schroeder, Norcliffe, Jordan, & Hainsworth, 2006a).

In 2002, two independent studies showed that leg crossing and muscle tensing – the physical counterpressure manoeuvres - during the prodromal phase of syncope could activate the skeletal muscle pump to raise blood pressure values enough, in order to control or even abort the syncopal episode (Brignole et al., 2002; Krediet, van Dijk, Linzer, Van Lieshout, & Wieling, 2002). These preliminary data led to a multicentre controlled study (van Dijk et al., 2006) of 223 patients with recurrent reflex syncope and recognisable prodromal symptoms, to evaluate the efficacy of the counterpressure manoeuvres. This trial showed that these manoeuvres, when performed immediately after the prodrome initiation, resulted in a reduction of up to 39% in the relative risk of recurrence of syncope. However, 35% of the patients randomised to perform these manoeuvres did not use them because they did not present a prodromal picture or

because the duration of the prodromes was not sufficiently long. Although the robustness of the evidence supporting this intervention is small, including the difficulty or even the impossibility to perform a blinded clinical trial to evaluate its effectiveness, considering its safety and the absence of associated cost, the counterpressure manoeuvres remain a valid option to consider in the initial therapeutic strategy of these patients (Shen et al., 2017b; Brignole et al., 2018). In our lab, and within the European Federation of Autonomic Societies (EFAS) scope, we provide information, animations and videos on digital support to patients, their caregivers, clinicians and other allied health professionals, regarding the various types of manoeuvres and the way how they should be performed to ensure maximum efficacy.

In relapsing cases, and despite merging in controversy, several clinical studies have pointed out "tilt training" - a program of autonomic modulation and adaptation- as a complementary plan to promote orthostatic tolerance (Vyas, Swaminathan, Zimmerman, & Olshansky, 2013). If symptoms persist, pharmacological treatment may then be necessary.

A. Role of the Orthostatic Training

At the end of the last decade of the 20th century, some observational studies in patients with recurrent syncope and a positive tilt testing response showed that the combination of in-hospital sequential tilt testing, complemented by a similar orthostatic manoeuvre performed daily at home, was effective in reducing syncopal recurrences (Ector, Reybrouck, Heidbuchel, Gewillig, & Van De Werf, 1998; Ector, Willems, Heidbüchel, & Reybrouck, 2005; Reybrouck, Heidbüchel, Van De Werf, & Ector, 2000, 2002; Abe, Sumiyoshi, Kohshi, & Nakashima, 2003; Abe, Kohshi, & Nakashima, 2005; Reybrouck & Ector, 2006; Tan et al., 2010). These results were confirmed through a recent meta-analysis of eight studies with five hundred patients, where it was shown that orthostatic training, also commonly called tilt training, was effective in preventing the recurrence of vasovagal syncope (n=500; OR 0.3; CI 0.15-0.61, $p<0.05$) (Vyas et al., 2013). Its long-

term effects should be carefully assessed, as tilt training requires a motivated and compliant patient, in particular after the first positive results.

In recent years, several investigators have performed randomized nonblinded trials with conflicting results (Foglia-Manzillo et al., 2004; Gurevitz et al., 2007; On, Park, Huh, & Soo Kim, 2007), which were mainly due to a low compliance of the patients allocated to tilt training, together with the myriad of different study protocols, difficult to compare between themselves. More recently, Tan and colleagues (Tan et al., 2010) published a blinded study of 22 patients in which the patients allocated to tilt training had fewer recurrences than those in the control group, who performed a sham manoeuvre. Nevertheless, doubts remain as to its efficacy due to the lack of demonstration of benefit in the recurrence of syncope in several other studies.

Physiological underlying mechanisms concerning the improvement in these patients' general condition are not yet entirely clear, and the working hypotheses include the desensitisation of cardiopulmonary receptors, autonomic remodelling and alterations in baroreceptor reflex, interference of humoral pathways the renin-angiotensin system and an increase of vasoconstrictor reserve (Verheyden, Ector, Aubert, & Reybrouck, 2008; J. Gajek, D. Zysko, S. Krzeminska, & W. Mazurek, 2009).

Thus, although some data support orthostatic modulation as a complementary plan to be included in the syncope treatment, more extensive studies and the understanding of its putative effects and their mechanisms are still a matter of study.

II. Pharmacological Treatments

Pharmacotherapy starts when conservative measures are inadequate, or when there are little or absent prodromes, and syncope is associated with severe bodily injury. However, drug therapy for vasovagal syncope remains highly controversial. Based on pathophysiologic mechanisms of reflex syncope, several drugs have been proposed and tested for treating patients with reflex syncope. Among them are beta-blockers,

disopyramide, scopolamine, theophylline, ephedrine, midodrine, clonidine, and serotonin reuptake inhibitors (Vyas et al., 2013; Brignole et al., 2018).

Several randomised clinical trials have analysed the role of beta-blockers, notably regarding metoprolol, pindolol, and atenolol (Madrid et al., 2001; Sheldon et al., 2006, 2012), and, overall, they did not show beneficial effects in patients with recurrent syncope. The largest randomised, controlled, double-blind trial conducted (the POST study) revealed that metoprolol was not effective in preventing recurrence of syncope at one-year follow-up (Sheldon et al., 2006). A subsequent meta-analysis, including the previously reported study, concluded that beta-blocker might be beneficial for patients aged >42 years when compared to patients <42 years (Sheldon et al., 2012). A multicentre, international, randomised, placebo-controlled study of metoprolol in the prevention of vasovagal syncope in patients > 40 years-old is underway, but the results have not yet been published (Raj et al., 2016).

Initial uncontrolled studies suggested that midodrine was useful in preventing syncope recurrences in patients with reflex syncope (Ward, Gray, Gilroy, & Kenny, 1998; Samniah, Sakaguchi, Lurie, Iskos, & Benditt, 2001; Perez-Lugones et al., 2001; Qingyou, Junbao, & Chaoshu, 2006). Indeed, in a meta-analysis including 115 patients from four studies, the recurrence of syncope was found to be lower and the quality of life superior during treatment with midodrine, when compared with non-pharmacological therapy or placebo (Liao et al., 2009). However, more recently, a randomised crossover study comparing midodrine vs placebo, in patients in whom nonpharmacologic therapy failed to prevent syncope recurrences, indicated that midodrine was not superior in preventing syncope recurrences in this population (Romme, van Dijk, Go-Schön, Reitsma, & Wieling, 2011). The results of the POST 4 study, a randomised clinical trial designed to evaluate the efficacy of midodrine compared to placebo, in preventing recurrence of syncope episodes are also awaited (Raj, Faris, McRae, & Sheldon, 2012).

Fludrocortisone was tested in a small, randomised, double-blind trial in children, and showed no benefits to its usage (Salim & Di Sessa, 2005); in a recent multicentre randomised trial of fludrocortisone versus placebo, in 210 patients with recurrent reflex

syncope, the authors showed a marginally nonsignificant reduction in syncope in the fludrocortisone cohort (Sheldon et al., 2016). However, when results were analysed after two weeks of dose stabilisation, there was a significant reduction in syncopal episodes between the two groups. As such, fludrocortisone may be reserved for patients with repeated episodes of vasovagal syncope and who do not present contraindications for its use, namely hypertension (Brignole et al., 2018).

The efficacy of the selective serotonin reuptake inhibitors on the management of refractory vasovagal syncope has also been analysed in three different controlled trials, with conflicting results (Di Girolamo, Di Iorio, Sabatini, et al., 1999; Takata et al., 2002; Theodorakis et al., 2006). There is still no evidence of the beneficial effect of serotonin reuptake inhibitors in preventing syncope recurrence (Brignole et al., 2018).

In summary, there is not yet enough evidence favouring the use of drugs for preventing syncope recurrences in patients with reflex syncope and truly effective drugs are still lacking. At the present time, pharmacological strategies should be reserved for patients with recurrent episodes that have a substantial impact on the quality of life and are associated with a high risk of trauma or for patients with high-risk activities concerning themselves or others.

III. Cardiac Pacing

In cases where non-pharmacological and pharmacological interventions prove to be insufficient to treat syncope patients, the use of a cardiac pacemaker has been proposed as a therapeutic strategy, although its benefit remains controversial in particular when applied to patients with non-cardio-inhibitory reflex syncope (Romme, Reitsma, et al., 2011; Varosy et al., 2017).

Initially, non-randomized and unblinded studies demonstrated a potential benefit of such intervention, with reduced recurrence of syncopal episodes. However, randomised, double-blind studies have shown mixed results (Flammang et al., 1999;

Richard et al., 2000; Connolly et al., 2003; Raviele et al., 2004; Brignole et al., 2012; Russo et al., 2013; Baron-Esquivias et al., 2017), with some of them favouring the use of cardiac pacing (ISSUE-3 (Brignole et al., 2012), Russo et al. (Russo et al., 2013), SPAIN (Baron-Esquivias et al., 2017)), with others not showing benefits for this intervention (SYNPACE (Raviele et al., 2004), VPS II (Connolly et al., 2003)). According to the most recent European Society of Cardiology guidelines, and in line with the ISSUE-3 and SPAIN studies, cardiac pacing implantation is currently considered an indication II-A for the patients aged ≥40 years with recurrent cardioinhibitory vasovagal syncope, with spontaneous documented symptomatic asystolic pauses (>3 seconds) or asymptomatic pauses >6 seconds due to sinus arrest, atrioventricular block, or the combination of the two (Brignole et al., 2018).

Discrepant results among the studies previously discussed have been actively debated and may be related to the following points: (1) heterogeneity in the inclusion criteria of the population; (2) differences in study designs; (3) different pacing modalities in the intervention arm; or (4) a genuine lack of benefit of cardiac pacing intervention in the vasovagal syncope patient. Lastly, the positive studies included a population that tended to be more aged, which raises an issue regarding the putatively different pathophysiological mechanisms of vasovagal syncope according to the age group, as well as their interactions with the ageing process itself.

Conclusions

Management of vasovagal syncope remains a crucial health-care challenge with vast implications. The wide range of treatment options and the debates concerning their efficacy often tend to leave the clinician constrained about the establishment of a rational treatment plan. In fact, at this stage, syncope treatment seems somewhat more of an art than a science.

CHAPTER 2

CHAPTER 2

PATHOPHYSIOLOGY OF VASOVAGAL SYNCOPE

Syncope has a wide range of clinical presentations with differences in trigger patterns, prodromal signs and symptoms, the onset speed, ictal phenomena and postictal events. All these depend not only on the cause of syncope but also on age and various other factors that affect clinical presentation. Ultimately, syncope results from a failure of the circulation to perfuse the brain, which means any factor affecting blood circulation may determine whether syncope occurs.

2.1. Cardiovascular responses to orthostatic stress

The adoption of upright posture provokes blood pressure homeostasis in two main ways. First, in the upright position, the cerebral arterial pressure is 15-30 mmHg lower than the one in the aortic arch. Moreover, the difference is even more relevant when compared with the one in a dependent arm (Van Lieshout, Wieling, Karemaker, & Secher, 2003; Claydon, Steeves, & Krassioukov, 2006a; Van Dijk & Wieling, 2013). Secondly, upon standing, 300-1000 ml of blood shifts into the venous capacitance system of lower extremities and the splanchnic area (Smit, Halliwill, Low, & Wieling, 1999; Mathias, 2002; Robertson, 2008). Additionally, the increase in transmural capillary pressure in the dependent circulation results in a prominent fluid filtration through capillary walls, into the extravascular tissue. This redistribution of blood decreases cardiac output, venous return and blood pressure; thus, the continuous maintenance of the upright posture demands the interaction between various cardiovascular regulatory systems (Stewart, 2012).

Cerebral perfusion is highly dependent on systemic arterial blood pressure (Mosqueda-Garcia, 2015a). Despite the above mentioned orthostatic-induced changes, mean arterial pressure at the cervical level is kept stable mainly through quickly acting neural reflex mechanisms, leading to a constriction of the capacitance vessels (Wieling & van

Lieshout, 1997). Initial adjustments to orthostatic stress are mainly moderated by autonomic adjustments significantly regulated by cardiovascular reflexes such as the baroreceptor reflex (Rowell, 1993).

The baroreceptor reflex is the dominant mechanism responsible for adjusting blood pressure, by rapid modulation of autonomic efferent function, leading to changes concerning cardiac output, heart rate, as well as total peripheral resistance (Mosqueda-Garcia, 2015a). It starts with the stimulation of arterial (high pressure) baroreceptors - mechanoreceptors located in the adventitia of the aortic arch and the carotid sinus - which are sensitive to beat-to-beat alterations in blood pressure. Then, baroreceptors respond to the deformation and distension of the vessel wall, originated by local changes in blood pressure. Afferent baroreceptor impulses from these zones travel through myelinated and unmyelinated nerve fibres within the carotid sinus and aortic nerves towards the medulla, particularly within the nucleus of the solitary tract (NTS) where the first synapse of the baroreflex can be found (Benarroch, 2012; Chapleau, 2012; Mosqueda-Garcia, 2015a). Efferent projections from the NTS deliver information to other autonomic centres, such as the ventrolateral medulla and the hypothalamic nuclei. These areas further integrate the baroreflex activity and give rise to the final autonomic efferent response that leads to adaptive alterations concerning heart rate and arterial blood pressure (Benarroch, 2012; Chapleau, 2012; Mosqueda-Garcia, 2015a). This “negative feedback” reflex mechanism keeps arterial blood pressure stable and intends to maintain adequate perfusion pressure in several organs, namely the brain (Eckberg, Harkins, Fritsch, Musgrave, & Gardner, 1986).

When blood pressure drops, arterial baroreceptors become unloaded, and the reduction in neuronal firing leads to an almost automatic decrease in parasympathetic activity, along with less release of acetylcholine from postganglionic parasympathetic fibres to Keith-Flack and Aschoff-Tawara nodes. Sympathetic activity increases 5 to 10 seconds later, being mainly mediated by norepinephrine released from postganglionic sympathetic fibres innervating the atrial, the ventricles and the blood vessel wall (Rowell, 1993). As a result, the immediate response to orthostatic stress is composed of tachycardia (parasympathetic withdrawal) and arterial vasoconstriction (sympathetic

activation). Changes in baroreceptor activity also affect breathing, as a result of the way central integration of visceral information is organised at the brainstem.

In spite of the fact that intact arterial baroreflexes are a fundamental mechanism in the first adjustments of blood pressure during orthostatic stress (Cooke et al., 1999), the relevance of reflex tachycardia on hemodynamic stability for extended periods of orthostasis is not so obvious (Convertino, 2014). In fact, there is some evidence suggesting that peripheral vasoconstriction adjustments are the fundamental components of medium-term blood pressure adjustments during orthostatic stress. Moreover, it has been hypothesized that the venous blood reservoir is the primary determinant, as the heart cannot pump blood that it does not receive (Rowell, 1993; Wieling, de Lange, & Jardine, 2014). We should also highlight that arterial vasoconstriction decreases blood flow to the venous section and diminishes peripheral venous pressure, originating passive elastic recoil of pooled venous blood from the lower limbs and splanchnic vasculature to the central circulation (Rothe, 1984).

In addition, continued orthostatic stress develops a group of neurohormonal changes reinforcing, in what concerns amplitude and time, the way cardiovascular reflexes act. In addition to the sustained increase in noradrenaline, a temporary increase in epinephrine, the renin-angiotensin-aldosterone system activation and discharges of arginine vasopressin have been noticed (Jacob et al., 1998). These additional responses originate various effects to maintain cardiovascular homeostasis, e.g., through direct vasoconstriction at the level of the vascular smooth muscle and through the increase of tubular Na^+ reabsorption in the kidneys, as to reduce the loss of body water (Jacob et al., 1998; Smit et al., 1999).

2.2. Pathophysiology of vasovagal syncope

Pathogenic mechanisms underlying vasovagal syncope are truly disparate, as well as still poorly understood. However, when analysing the pathogenesis of syncopal conditions, it is crucial to differentiate the mechanisms that originate syncope and the predisposing factors (Brignole et al., 2018). It has been admitted for a while that, in what concerns

susceptible individuals, distinct stimuli may lead to the development of vasovagal episodes (Mosqueda-Garcia, Furlan, Tank, & Fernandez-Violante, 2000; Mosqueda-Garcia, 2015b). As previously stated, in this work the focus is set on orthostatic vasovagal syncope. Although the exact pathophysiology of vasovagal syncope gives rise to different views on the subject, several implied mechanisms have been unveiled (Grubb, 2005).

Vasovagal syncope originated from orthostatic stress stems from a disruption of the normal reflex response to gravitational forces (Mosqueda-Garcia, 2015b). According to the Sharpey-Schafer model (Sharpey-Schafer, 1956; Wieling et al., 2016), some authors have implied that when there are elements leading to decreased venous return and cardiac filling, three main conditions leading to vasovagal syncope arise (Mark, 1983; Kosinski, Grubb, & Temesy-Armos, 1995; Fenton, Hammill, Rea, Low, & Shen, 2000): (1) a more significant reflex increase in sympathetic tone to the heart, resulting in positive chronotropic and inotropic cardiac impact, (2) the development of ventricular hypovolemia along with an increased cardiac sympathetic stimulation originating strong contraction of the volume-depleted ventricle (Kosinski et al., 1995; Fenton et al., 2000); also, (3) the sharp contraction of the hypovolemic ventricle, in turn, distorts ventricular muscle, stimulating “ventricular” afferents in the left ventricle. This may provoke an inhibitory response, alike to the one of Bezold-Jarisch reflex, with a paradoxical decline of peripheral sympathetic tone and the increase in vagal tone, resulting in vasodilatation and bradycardia (Mark, 1983) . These three elements constitute the basis of the “ventricular theory” concerning vasovagal syncope development (ÖBerg & Thorén, 1972; Mosqueda-Garcia, 2015b).

This theory was based on the observation by ÖBerg and Thoren that some ventricular non-myelinated afferents in the cat became excited under the referred conditions (ÖBerg & Thorén, 1972). In fact, the “ventricular theory” for vasovagal syncope was widely accepted since it came up with an explanation for several clinical observations (e.g., exertional syncope in patients with aortic stenosis). Moreover, it provided a rational basis for the use of beta-adrenergic agonists on the diagnosis (isoproterenol

plus tilt table testing) or in the treatment (beta-adrenergic antagonists) of vasovagal syncope (Mosqueda-Garcia, 2015b).

Nevertheless, some studies have revealed reservations relatively to this theory (Mosqueda-Garcia, 2015b). First of all, extreme sympathetic overstimulation has been called into question by observations that have found normal (Sra et al., 1994) or even decreased (Goldstein et al., 1982; Jacobs et al., 1995; Kaufmann et al., 1995; Mosqueda-Garcia et al., 1997) plasma norepinephrine levels at the moments which precede syncope. Furthermore, a reduced maximal increase in Muscle Sympathetic Nerve Activity (MSNA) has been noticed in patients developing vasovagal syncope (Mosqueda-Garcia et al., 1997; Béchir et al., 2003; Gautam Vaddadi, Esler, Dawood, & Lambert, 2010; Lambert & Lambert, 2014). In addition, the second condition has also been put into question since echocardiographic measurements during head-up tilt have shown that the heart is not necessarily near-empty, nor powerfully contracting (Yamanouchi et al., 1996; Novak, Honos, & Schondorf, 1996). Lastly, the third condition, “ventricular heart afferents initiating the vasovagal response”, has been put into question both in animal studies and in human patients with cardiac transplantation. Concerning animal studies, surgical denervation of the heart did not avoid sudden sympathetic withdrawal during sudden blood volume decrease (Morita & Vatner, 1985; Scherrer, Vissing, Morgan, Victor, & Hanson, 1990). Moreover, neurocardiogenic hypotension and bradycardia during orthostatic stress were noticed in patients who had gone through orthotopic heart transplant (Fitzpatrick, Banner, Cheng, Yacoub, & Sutton, 1993; Lightfoot, Rowe, & Fortney, 1993; Grubb, 2005). Additionally, the Bezold-Jarisch reflex is not obtained by mechanical stimulation. On the contrary, it is the result of chemical stimulation (Clozel, Pisarri, Coleridge, & Coleridge, 1990). To sum up, all the evidence presented above casts severe questions on the validity of the “ventricular theory” for the development of vasovagal syncope, while upholding the engagement of other mechanisms in the evolution of this syndrome (Mosqueda-Garcia et al., 2000; Mosqueda-Garcia, 2015b).

I. The role of the autonomic nervous system in syncope

Four stages of the cardiovascular responses resulting in syncope have been presented: 1) early stabilisation, 2) circulatory instability, 3) terminal hypotension and 4) recovery (Julu, Cooper, Hansen, & Hainsworth, 2003; Jardine et al., 2018). The duration of the phases can vary considerably. Nevertheless, all of them may be partly acknowledged in most of the patients.

In the beginning, the term "vasovagal" was used when referring to the fainting process due to the perception that parasympathetic activity was prevailing (Lewis, 1932; Grubb, 2005). Although parasympathetic activity seems to expand during syncope (being the cause for the bradycardia), the main responsible for the loss of consciousness is vasodilatation, which leads to hypotension (Rea & Thames, 1993). Bradycardia is usually relatively unimportant as heart rate is rarely considerably decelerated (Hainsworth, 2003). Moreover, prevention of bradycardia by pacing the heart does not always avoid or delay syncope (Lewis, 1932; Sra et al., 1993; Hainsworth, 2003).

The reduction of blood pressure has been found to have a relationship with either an active or a passive neural output or with neuro-humoral mechanisms (Mosqueda-Garcia et al., 2000; Mosqueda-Garcia, 2015b). Concerning the active neural mechanisms, the activation of the sympathetic cholinergic vasodilator mechanism has been supported (Willems & Bogaert, 1978; Halliwill, Dietz, & Joyner, 1996; Wong & Hollowed, 2016). However, failure to prevent vasodilation by intra-arterial cholinergic blockade has been reported, which may represent an argument against this mechanism (Barcroft, Brod, Hejl, Hirsjarvi, & Kitchin, 1960).

On the other hand, evidence for a passive vasodilation mechanism for vasovagal syncope appears to be more supported by experimental evidence (Mosqueda-Garcia, 2015b). The most credible note comes from recordings of human sympathetic nerve traffic in subjects who have gone through vasovagal episodes (Wallin & Sundlöf, 1982; Dietz et al., 1997; Mosqueda-Garcia et al., 1997, 1998; Ellenbogen et al., 2006). In some of these studies, the reflex rise in muscle sympathetic nerve activity seems to be blunted

in susceptible individuals. With the extension of the precipitating factor, MSNA falls step by step until its total disappearance, a few seconds before syncope (Mosqueda-Garcia et al., 1997, 1998). Nonetheless, other authors have stated that MSNA does not vanish during vasovagal syncope (Vaddadi et al., 2010), which may indicate that, in some cases, the pathogenesis of this syndrome involves alternative mechanisms.

At present, it is discussed whether vasodilation is the dominant hypotensive mechanism preceding vasovagal syncope. In fact, some studies have implied that the decrease in cardiac output, rather than vasodilation, might be the leading cause for hypotension (Gisolf et al., 2004; Verheyden, Liu, et al., 2008; Murrell et al., 2009; Fu, Verheyden, Wieling, & Levine, 2012; Fu & Levine, 2014; Wieling et al., 2016). For instance, it has been reported that although every subject demonstrated an initial reduction concerning cardiac output during head-up tilt, patients who became hypotensive showed a further decline later on during the manoeuvre relatively to this factor (Jardine et al., 2002). Other authors hold to the theory that some neuro-humoral mechanisms, namely a 'sympathoadrenal imbalance', are the *de facto* cause of vasovagal syncopal attacks. This theory arose from data evidencing an increase of both norepinephrine and epinephrine levels during the first phases of upright tilt-induced syncope (Sra et al., 1991). In another set of studies, patients with reflex syncope revealed an abnormal tonic decrease of cardiac norepinephrine release, which may imply that vasovagal syncope involves both tonic restraint of cardiac sympathetic outflow and reduced increases in sympathetic outflow to skeletal muscle during orthostasis (Goldstein et al., 2002, 2003). In these studies, patients with head-up tilt-induced syncope revealed noticeable progressive increases, up to 11 times, of the baseline values of plasma epinephrine levels. At the same time, norepinephrine rose to a much lower degree than epinephrine, generating a "sympathoadrenal imbalance." In the same patients, forearm vascular resistance decreased by 21% before syncope, suggesting that sympathoadrenal imbalance precedes tilt-evoked and spontaneous reflex syncope and is directly related to simultaneous skeletal muscle vasodilation. This way, the sympathoadrenal imbalance appears to have a contribution to the hemodynamic changes leading to reflex syncope. Hence, the initial fall in central blood volume as a result of peripheral venous pooling originates a reflex increase in sympathetic output with the appearance of tachycardia

and vasoconstriction, appearing to try to keep a reasonable haemodynamic condition. However, this increase in sympathetic tone can simultaneously sensitise and ease the activity of the cardiac mechanoreceptors involved in the production of vasovagal events (Waxman, Asta, Cameron, & Endrenyi, 1992; Grubb, 2005).

II. Baroreceptor reflex dysfunction in Vasovagal Syncope

Disruption of the baroreceptor reflex seems to be a feature of the vasovagal syncope since it is clear from the concurrent development of hypotension and bradycardia. In vasovagal syncope, baroreceptor mechanisms seem to be nullified and not capable of preventing the low pressure and/or heart rate levels that give rise to cerebral hypoperfusion and can lead to syncope (Mosqueda-Garcia et al., 1997, 2000; Mosqueda-Garcia, 2015a).

Many authors have supported the idea that modified or faulty baroreflex function is a primary mechanism concerning the vasovagal syncope. Evidence for increased sensitivity (Wahbha, Morley, Al-Shamma, & Hainsworth, 1989; Adler, France, & Ditto, 1991; Sneddon, Counihan, et al., 1993a; Pitzalis et al., 2003) or decreased function (Thomson, Wright, Frenneaux, & Fernandez-Violante, 1997; Wijeysundera et al., 2001; Ogoh, Volianitis, Raven, & Secher, 2004; Samniah, Sakaguchi, Ermis, Lurie, & Benditt, 2004; Iacoviello et al., 2008; Meyer et al., 2010) has been presented, and, consequently, theories have been advanced.

An increasing number of observations and well-conceived experimental investigations have revealed a reduction in baroreflex sensitivity or function as an aetiological condition for vasovagal syncope. Several patients with recurrent vasovagal syncope, which was confirmed with repeated positive tilt table tests, showed an evident decline in baroreflex function, supported by a depression in the reflex sensitivity and effectiveness index values when compared to control subjects (Iacoviello et al., 2008). Therefore, even at low stimulation levels, baroreflex function, including its sensitivity, is

reduced in subjects with vasovagal syncope. This reduction probably constitutes an etiological factor (Ellenbogen et al., 2006). However, some authors have implied that baroreflex function is retained but then suddenly suppressed by a depressor reflex with its origin in the heart (van Lieshout, Wieling, Karemaker, & Eckberg, 1991; Ogoh et al., 2004).

Overall, most of the available studies report some autonomic and, in particular, baroreceptor reflex dysfunction in subjects with vasovagal syncope, which leads to the inability to sense or compensate for changes in gravitational forces. The extent and the exact type of dysfunction remain to be thoroughly characterized.

2.3. Conclusions

In summary, there are multiple mechanistic pathways to syncope, and our patient assessments and treatments should reflect them. A lot more has to be studied and understood concerning the role of the autonomic nervous system in syncope. At present, more in-depth knowledge is still needed to comprehend why some people have syncopal attacks, while others do not, and why some have frequent recurrences while others only faint sporadically. Also, an adequate treatment strategy for patients with frequent syncopal recurrences is still required, as no effective treatments are currently available.

CHAPTER 3

CHAPTER 3

SPECIFIC AIMS AND WORKING HYPOTHESIS

I. Overall purpose of the present PhD thesis

Syncope is a significant and common clinical problem, often disabling, causing injury and being associated with meaningful healthcare and societal costs. Recurrent syncope has severe effects on the patient's quality of life, inducing impairment in lifestyle, comparable with that associated with prevailing chronic illnesses such as arthritis, depressive disorders and renal disease.

Reflex syncope classically refers to a heterogeneous group of conditions in which cardiovascular reflexes regulating circulation become intermittently inappropriate in response to a trigger. Despite its frequent occurrence, reflex syncope's pathophysiology is still an enigma regarding what triggers the downward spiral of events that ultimately lead to hypotension, bradycardia and loss of consciousness. Various studies have investigated the pathophysiology of syncope in individuals prone to faint and the underlying physiological constraints in healthy subjects, but the understanding of vasovagal syncope's pathophysiological mechanisms is still a challenge. Autonomic dysfunction of various extents has been pointed out as a putative factor leading to syncopal events. Nevertheless, the evidence in the literature is conflicting about the role of the efferent autonomic activity in vasovagal syncope's genesis, as well as regarding the behaviour of autonomic reflexes in syncope patients.

Although the diagnosis of reflex syncope can be achieved from a patient's history combined with tilt table testing, a negative HUT response does not exclude the diagnosis, bringing out concerns about HUT usefulness, sensitivity and specificity.

The management of reflex syncope is multifactorial and complex. It involves various types of treatment strategies due to the multiple pathophysiological mechanisms that have been described. They also involve patient education and guidance to the avoidance of putative syncopal stimulus, the usage of physical counter-pressure measures, the increase of water and salt ingestion, the adherence to drug treatments, and autonomic modulation procedures. Regrettably, there are no widely agreed-on specific treatments, and the benefits of drug therapy are often disappointing. Autonomic modulation through orthostatic training may be an effective way of syncope management; however, up to the moment, the available results do not support its full acceptance in clinical practice.

Thus, the general purpose of the present work is to contribute with the required skills and resources for the understanding of the functional mechanisms underlying reflex syncope and their translation into clinical practice, as well as to contribute to the prediction of syncopal episodes and the stratification and treatment of these patients. In accordance, our aims are:

1. To design and implement new mathematical and computational tools based on hemodynamic and autonomic variables able to

    a. Complement, together with the clinical evaluation, the understanding of the mechanisms underlying reflex syncope, particularly the dynamicity of the pre- and post-syncopal periods;

    b. Refine diagnostic accuracy, sensitivity and specificity of the head-up tilt-table test;

    c. Predict an impending episode of reflex syncope.

2. To establish an effective and personalised treatment option to patients with frequent reflex syncope, refractory to conventional treatment options, contributing to an increase in quality of life and a reduction of health care and associated costs.

With our results, we expect to generate new knowledge, which will contribute to filling the gap between the best available scientific information and its application in clinical practice, by tackling the three pillars of translational medicine: benchside, bedside and community.

CHAPTER 4

CHAPTER 4

COMPUTATIONAL TOOLS FOR ASSESSING CARDIOVASCULAR VARIABILITY

Exploring the hypothesis under study...

Reflex syncope is defined as a transient loss of consciousness due to an abrupt fall in brain perfusion and autonomic dysregulation, even temporary, has been implicated in the pathophysiology of syncope.

The autonomic nervous system has primary biological importance as it controls, in an exact way, almost all bodily functions, being its balanced functioning crucial for our life and well-being. Thus, when it fails due to a functional defect, a lesion on its neuronal network or due to ageing, an autonomic failure is installed. In these conditions, clinical evaluation is the most crucial step in approaching the patient with autonomic dysfunction. Bearing that in mind, a directed and comprehensive history and examination, together with specialized autonomic testing, should be included in the clinical algorithm for the evaluation of these patients.

Autonomic laboratory evaluation helps to acknowledge whether the autonomic function is healthy or not and, if abnormal, to indicate the local and degree of functional deficit of the dysfunction, as well as if the autonomic failure is of primary or secondary origin. Most of the manoeuvres used to evaluate autonomic function address changes in cardiovascular parameters, such as heart rate and blood pressure. There are several provocative manoeuvres which can be performed, taking into consideration the patients and their condition, the suspected autonomic dysfunction and the available equipment. Data analysis follows the same profile, depending on the laboratory expertise and equipment used for data collection; however, in general, it is implemented in the time, frequency or time-frequency domains.

In recent years, various algorithms to evaluate autonomic dysfunction were launched onto the market, based on the circadian changes of physiological parameters. Nevertheless, most of these algorithms are based on the analysis of beat-to-beat rhythmic changes of heart rate in stationary conditions, analysed on the frequency domain using the Fast Fourier Transform (FFT). However, the response of the autonomic nervous system to a provocative stimulus is of quick onset and short duration, showing high dynamicity, which is one, if not the major, of its intrinsic properties as an adaptative system and homeostasis maintainer. Thus, new and integrative tools are needed for clinical autonomic evaluation.

In accordance, as a working hypothesis, we establish that

A new tool, addressing more than one physiological parameter, the development of new algorithms on the time-scale domain and the integration of various methodologies of data analysis will improve medical decision-making regarding diagnosis, therapy and prognosis of autonomic impairment, by providing a rapid patient-tailored evaluation of the autonomic nervous system function.

I. Introduction

In physiological terms, the ANS, jointly with the endocrine and the immune systems, contributes to the maintenance of homeostasis by controlling every visceral function. Together with a detailed history and physical examination, ANS testing is essential for the evaluation of various clinical conditions, to the establishment of an effective and personalised therapeutic programme and to follow-up patients. Among various possibilities, provocative manoeuvres addressing cardio-respiratory parameters and heart rate variability methods, are the most common methods to evaluate autonomic tone.

Fluctuations in heart rate were first recorded by ancient physicians (Samaan, 1935; Lacey & Lacey, 1958; Hon & Lee, 1963; Malik et al., 1996; Berntson et al., 1997; Cheng, 2000). Only recently, these new methods have received increased clinical interest, with

the advance of computer techniques, that allow for parsing heart rate variability (HRV) into components which potentially yield information about the autonomic control of cardiac activity. However, these technical advances have not resulted in bedside application of HRV analysis, and evidence of their usefulness in real-world clinical practice is still limited (Sosnowski, 2010).

The works of Hon and Lee (Hon & Lee, 1963) and Ewing (Ewing & Clarke, 1982), are a breakthrough in understanding autonomic function. After these studies, the usage of non-invasive methodologies to evaluate ANS has increased, and both time- and frequency domain methods have been used to analyse physiological signals variability. Despite a vast multitude of different algorithms in different domains, the most usual processing methods are the Fast Fourier Transform (FFT) or the autoregressive spectral analysis. The FFT decomposes signals into a series of sine and cosine functions of different frequencies and amplitudes, allowing the definition of a power spectrum. Three significant ranges of frequencies for human subjects can be recognised: *very low frequencies (VLF, 0–0.04Hz), low frequencies (LF, 0.04–0.15Hz) and high frequencies (HF, 0.15–0.4 Hz)* (Malik et al., 1996). Concerning the physiological significance of LF and HF bands, from pharmacological studies (Akselrod et al., 1981, 1985), it became clear that the HF band is under control of the parasympathetic system and is related to the respiratory rhythm. The full significance and origin of the LF band are not yet completely understood but, according to Malliani (Malliani, 2000), LF characterises sympathetic excitation, regardless of its genesis, with a putative metabolic influence. Another index, the LF/HF ratio, is also relevant since it allows the quantification of the relative balance between the two branches of the ANS in certain physiological or pathophysiological conditions (Malliani, 2000).

The application of FFT has, nonetheless, significant limitations (Parati et al., 1990; Duhamel & Vetterli, 1990), such as the requirement of a stationary signal and a long period of data collection (of at least 5 minutes), making it an inappropriate tool for the evaluation of the rapid autonomic adaptations, which are the essence of the autonomic control. Physiological systems and signals are associated with constant adaptative changes, in order to keep homeostasis, this being the main reason why attention is paid

to the nature and structure of these time variations, so as to understand the physiological phenomena. Frequency domain analysis has thus been used as a powerful tool to break physiological constituents into their components, but physiological data have a non-linear nature, and its analysis requires short time series recordings. Short-time Fourier transform (STFT) has been proposed to overcome these limitations, (Elsenbruch, Wang, Orr, & Chen, 2000) but it still has mathematical constraints, due to the employment of a window with fixed time and frequency resolutions.

As stated above, there are several laboratory methods used in physiology and medicine to investigate autonomic function, mainly cardiovascular function, since blood pressure (BP) and heart rate (HR) are quickly recorded, painless and non-invasive for patients. However, from our knowledge, there is not available a validated platform, capable of integrating all relevant heart rate and blood pressure variability analysis tools.

This way, there is the need for the development and validation of new mathematical tools, capable of dynamic autonomic evaluation, and for a user-friendly and modular computational tool that incorporates time, frequency and time-frequency analysis of biological signals, to be used in physiology and medicine. In fact, both needs are the purpose of the present work.

4.1 A time-scale tool based on discrete wavelet transform to evaluate rapid autonomic adaptations

Wavelet analysis, a mathematical function used to divide a continuous-time signal into different scale components, has been proposed as a method to overcome FFT's limitations (Wiklund, Akay, Morrison, & Niklasson, 2002; van den Berg, Neely, Wiklund, & Landström, 2005; Urbančič-Rovan, Bernjak, Stefanovska, Ažman-Juvan, & Kocijančič, 2006; Mallat, 2009). With discrete wavelet transform (DWT), a time-scale analysis can be performed, allowing the visualisation of the beat-by-beat contributions of LF and HF to the observed changes of a signal, in a specific time point.

Wavelets are oscillating mathematical functions of short duration, which separate a signal into its different component frequencies and then analyse each of them, with a resolution matching its scale. The signal is decomposed into approximation and details, at several scales. There are several types of wavelet functions, but those which appear most suitable for blood pressure and heart rate signal profiles belong to the Daubechies family. The wavelet function can be defined mathematically by (**1**), following (Addison, 2002; Goswami & Chan, 2011)

$$\psi_{a,b}(t) = \frac{1}{\sqrt{|a|}}\psi\left(\frac{t-b}{a}\right) \quad \textbf{(1)}$$

where $\psi_{a,b}$ is the wavelet functions, with $\psi_{1,0}$ being the mother wavelet and the remaining one being the daughter (often also called child) wavelets. The *a* parameter represents the change of scale, whereas parameter *b,* which is a real number, conveys the translation in the time axis *(t)*. The parameter $\frac{1}{\sqrt{|a|}}$ is the normalisation factor that ensures that the energy of the wavelet is independent of *a* and *b*. We can then define the wavelet transform as (**2**)

$$W(a,b) = \int_{-\infty}^{\infty} f(t)\frac{1}{\sqrt{|a|}}\varphi\left(\frac{t-b}{a}\right)dt \quad \textbf{(2)}$$

Ingrid Daubechies has developed a group of widely used discrete wavelets, the Daubechies (Daubechies, 1992). Her definition allows for the construction of discrete wavelet transform, defined as:

$$\psi_{a,b}(t) = 2^{-\frac{m}{2}}\psi(2^{-\mathrm{m}}\mathrm{t} - \mathrm{n}) \quad \textbf{(3)}$$

where *a* and *b* control the dilation and translation, respectively. In many applications, the computation of the wavelet transform implies that the original signal crosses a series of high-pass filters and low pass filters (**4**)

$$\emptyset_{m+1,n}(t) = \frac{1}{\sqrt{2}}\sum_{k} c_k \emptyset_{m,2n+k}(t)$$
$$\psi_{m+1,n}(t) = \frac{1}{\sqrt{2}}\sum_{k} b_k \emptyset_{m,2n+k}(t) \quad \textbf{(4)}$$

where *Ø(t)* is the scaling function, *ψ(t)* is the wavelet function, c_k are the coefficients of scale, b_k the wavelet coefficients and *k* is the index of the location of transform coefficients. As a result of high pass filters, detail coefficients are obtained, while low-pass filters return the coefficients of approximation (**5**):

$$S_{m+1,n}(t) = \frac{1}{\sqrt{2}}\sum_{k} c_{k-2n} S_{m,k}$$
$$T_{m+1,n}(t) = \frac{1}{\sqrt{2}}\sum_{k} b_{k-2n} S_{m,k} \quad \textbf{(5)}$$

The components of approximation (A) and detail (D) are obtained by reconstructing the coefficients of approximation and detail (Addison, 2002), where *M* is the last level of decomposition.

$$A_M(t) = S_{M,n}\emptyset_{M,n}(t)$$
$$D_m(t) = \sum_{n=0}^{2^{M-m}-1} T_{M,n}\psi_{m,n}(t) \quad \textbf{(6)}$$

In these operations, at each level of decomposition, half of the frequencies of the original signal are removed, according to Nyquist's rule. Consequently, the results of the filters are sub-sampled by a factor of 2. This decomposition causes a decrease in the temporal resolution in the order of 2x but can double resolution in the frequency domain. One can then rewrite the original signal X, using individual components approaches and details (**7**):

$$X = A_M(t) + \sum_{m=1}^{M} D_m(t)\,, \qquad m = 1,2,\dots,M \tag{7}$$

Determination of the energy is possible using the components approach (A) and detail (D) with the mean square root (**8**):

$$A_{rms,m} = \sqrt{\frac{1}{N}\sum_{r}|A_m(r)|^2}$$
$$D_{rms,m} = \sqrt{\frac{1}{N}\sum_{r}|D_m(r)|^2} \tag{8}$$

where *r* is the total number of data in the *n-th* level.

The total energy can then be calculated by (**9**):

$$\sum_{r=1}^{N}|s(r)|^2 = \sum_{r=1}^{N}\left|A_{rms,M}(r)\right|^2 + \sum_{m=1}^{M}\sum_{r=1}^{N}\left|D_{rms,m}(r)\right|^2 \tag{9}$$

Herein, we describe the usefulness of wavelet analysis for the investigation of the autonomic function applied to the acute changes of BP and HR signals recorded during standard autonomic manoeuvres, including the cold pressor test (CPT), deep breathing (DB) and the Valsalva manoeuvre (VM).

II. Methods

A. Inclusion and Exclusion Criteria

Seventeen healthy volunteers (14 females and three males) with a mean age of 44±13 (range 20-64) years old were included in this study. None of these subjects had clinical signs of cardiovascular, neurological or metabolic disorders, and none was under

medication. Tests were performed in a dedicated autonomic laboratory, in a quiet environment, with controlled temperature and humidity, during the morning, after a light breakfast, without ingestion of caffeine or other xanthines. Alcohol and tobacco were not allowed in the previous day and on the test day. Some subjects were unable to perform all the tests adequately, so these results have been withdrawn. Accordingly, the cold pressor test was validated in only 15 of the 17 subjects (12 females and three males, mean age 42±14 years) and the Valsalva manoeuvre and deep-breathing tests in 14 of the 17 subjects (three females and 11 males, mean age 43±13 years). Arterial blood pressure and ECG were continuously monitored using a Task Force Monitor (TFM) model 3040i (CNSystems, Graz, Austria). Studies were approved by the Ethics Committee of the Faculty of Medicine of Lisbon and performed under informed consent, according to the Declaration of Helsinki and the Oviedo Convention.

B. ANS experimental protocol

The following standardised ANS evaluation protocol was developed: after a resting period of 15 minutes in the supine position, the subject's right hand was immersed in ice-cold water (4^{o}C) for 1 minute. The subject was instructed to breathe regularly and to avoid sustained inspiration, which could mimic a Valsalva manoeuvre. After a second resting period of 15 minutes in a sitting position, the subject was instructed to breathe, for 15 seconds, against a constant pressure of 40 mmHg after a deep inspiration (Valsalva manoeuvre). After this manoeuvre, a final rest period of 15 minutes was allowed to elapse before the same subject was instructed to breathe deeply at a rate of 6 breaths/min, guided by a metronome, for a period of 1 minute (deep metronomic breathing).

C. Signal acquisition and processing

All data were acquired at 1 kHz and were analysed on the time-frequency domain using discrete wavelet transform (DWT) through Daubechies 12 (Db12). These orthogonal

wavelets were selected because the shape of this wavelet approaches the type of feature present in the recorded time series.

Data were sequentially processed in OriginPro 8 (Origin Lab Corporation, Northampton, MA, USA). A peak-to-peak routine was implemented in order to detect the highest values of blood pressure and QRS fiducial points, and to further reconstruct the time evolution curve of systolic blood pressure (sBP) and heart period - RR intervals (RRI), which were then computed in an Matlab environment (Matlab, MathWorks, Natick, MA, USA). The RRI and sBP were, then, interpolated by a cubic spline and resampled (resampling period=0.193 s) to ensure the matching between the centre of the frequency range of interest and the central frequency, associated to one of the scales of the DWT analysis. The Matlab Wavelet Toolbox was used to implement the DWT Db12 analysis of the resampled data.

Briefly, the resampled RRI and sBP time series were decomposed ($f_{RR(t)}$ and $f_{sBP(t)}$) into a sum of details and approximation at different scales of resolution. The central frequency associated with each scale, f_C, was calculated by $f_C = \frac{F_c}{a\Delta}$, where F_c is the central frequency of the used wavelet, a is equal to 2^{-j} where the scale is j and Δ is the resampling period. The signal was decomposed into 12 scales. The wavelet transform of the analysed signals at scale j and position μ is computed using the following relation:

$$Wf(\mu, j) = \int_{-\infty}^{+\infty} f(t) \frac{1}{\sqrt{2^j}} \Psi\left(\frac{t-\mu}{2^j}\right) dt$$

where ψ is a wavelet function, W f (μ, j) are the wavelet coefficients at scales and $f_{(t)}$ either $f_{RR(t)}$ or $f_{sBP(t)}$.

The selected wavelet coefficients for each detail relate to signal frequencies between 0.04–0.15 Hz and 0.15–0.4Hz (LF and HF, respectively; (Malik et al., 1996). The square of the details amplitude (SqA) was then calculated and, for a specified interval of time, LF and HF values were considered as the average of SqA across the details associated

with the frequency ranges of interest. Wavelet analysis was performed on data collected at different periods, according to the following protocol of data analysis.

D. Data Collection and Analysis

Data were analysed using discrete wavelet transform (Db12), applied to sBP and RRI, derived from arterial BP and ECG, respectively. For each autonomic test, data analysis was carried out as follows.

Cold Pressure Test (CPT)

The analysis of the cardiovascular variables was done during two periods: (1) the last 2 minutes of the resting period, just prior to the test (CTR); and (2) for 1 minute, divided into six epochs of 10 seconds. The period of 10 seconds with the most significant change from maximum to minimum was the one chosen to compare with CTR.

Valsalva Manoeuvre (VM)

The analysis of sBP and RRI was performed in three periods: (1) the last minute of the resting period, just prior to the test (CTR); (2) during 15 seconds of the Valsalva manoeuvre (VM); and (3) during the next 35 seconds after VM (postVM).

Deep Breathing Test (DB)

Data analysis was carried out in two periods: (1) the last minute of the resting period just prior to the test (CTR); (2) during 1 minute of DB, divided into six epochs of 10 seconds. The period of 10 seconds that had the most substantial change from maximum to minimum was the one chosen to compare with CTR.

Statistical Analysis

For CPT and DB, statistical analysis of the differences of LF, HF and LF/HF between the mean of control values and the mean values of each period of analysis was carried out using Student's t-test and differences were considered significant when $P < 0.05$.

Statistical analysis for VM data was performed using repeated ANOVA (Bonferroni test). All data were expressed as means ± SD.

III. Results

A. Physiological profiles of cardiovascular signals in healthy subjects

The physiological profiles presented upon each manoeuvre reflected the expected autonomic response to the triggering in a healthy individual, under a specific age group.

The cold pressor test unleashed the typical progressive increase of blood pressure that persisted during the time of immersion as expected in a healthy subject (Figure 4, left panels).

The sustained expiratory effort or strain against a closed glottis, which is the core of the Valsalva manoeuvre, evoked the appropriate modifications in blood pressure and heart rate evidencing the four defined phases of this manoeuvre. In p*hase I*, due to the strain onset, blood pressure increased, and a decreased heart rate was also observed. During *phase II*, the first reflex phase, a sharp decrease in blood pressure, to a level below baseline was followed by a rise in pressure and tachycardia. In *phase III*, a slight decrease in arterial pressure, related to the strain release, and an increase in heart rate were documented; in *phase IV*, the second reflex phase, an overshoot of blood pressure and bradycardia were noted (Figure 3).

Upon the deep breathing test, the typical modulation of heart rate by respiration was shown (Figure 6, top panel), without significant changes in blood pressure values.

A. Wavelet Analysis of Systolic Blood Pressure and R–R intervals

On cold pressor test, the observed cardiovascular changes were related to an increase in LF_{SBP} and LF/HF_{SBP} (Figure 4, right panels).

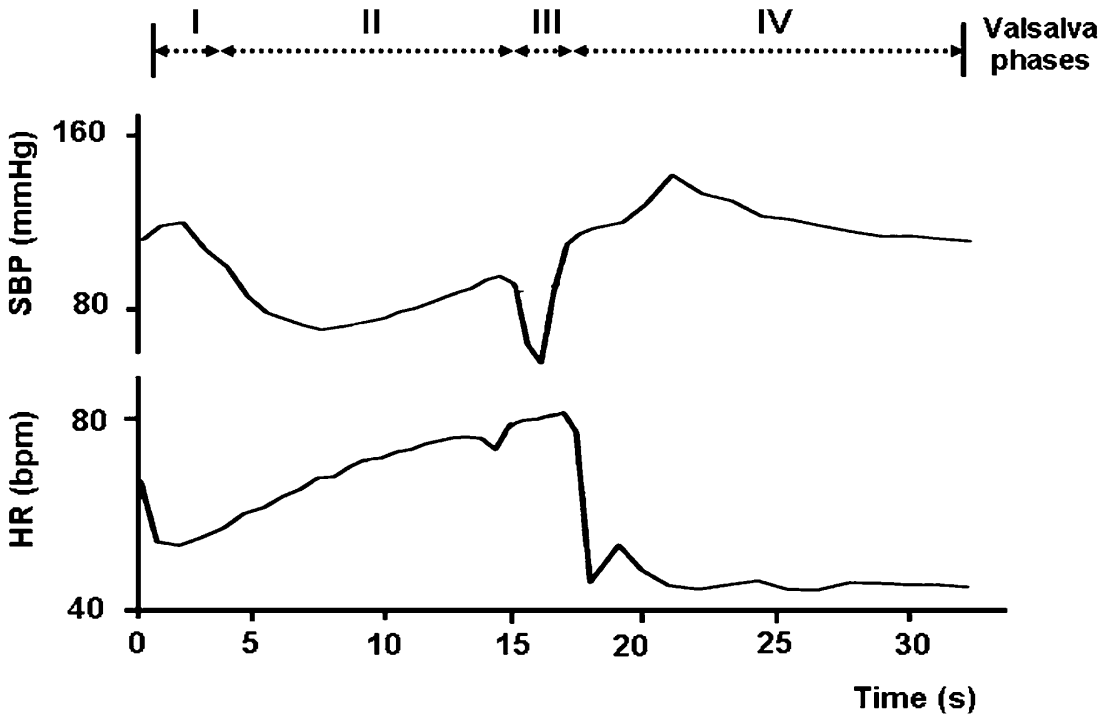


Figure 3. Systolic blood pressure (sBP) and heart rate (HR) changes for a healthy subject during the Valsalva manoeuvre (VM).

The four characteristic phases of VM are shown: phase I, an increase in blood pressure owing to the onset of the strain and a decrease in heart rate; phase II, a sharp decrease in blood pressure below baseline levels during the maintenance of the strain followed by an increase in pressure accompanied by an increase in heart rate; phase III, a short decrease in arterial pressure related to the release of the strain and an increase in heart rate; and phase IV, an overshoot of blood pressure and bradycardia.

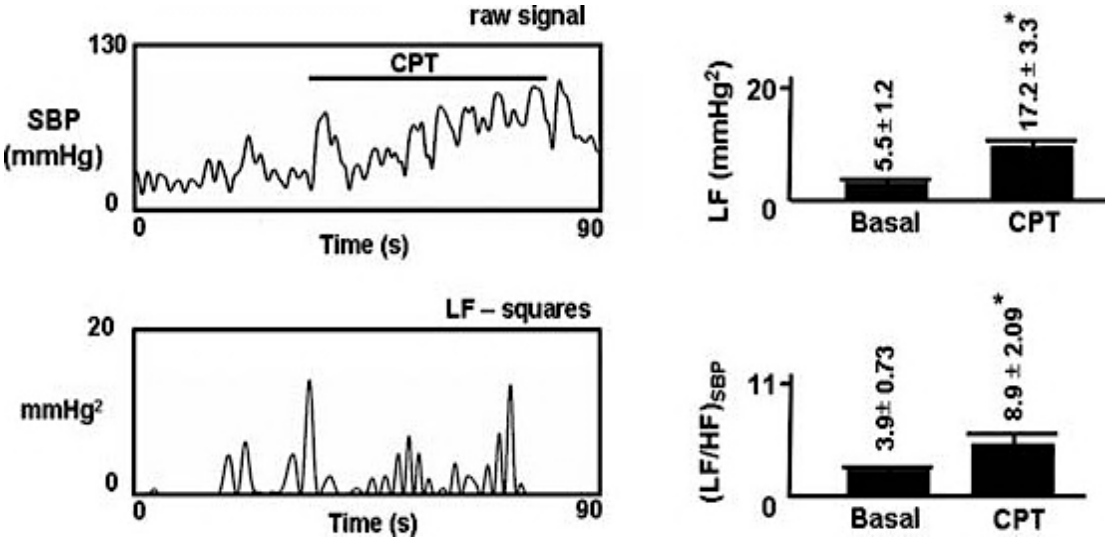


Figure 4. Wavelet analysis of systolic blood pressure changes during the cold pressor test.

The top left panel shows a raw signal of systolic blood pressure, illustrating the progressive increase in pressure during the immersion of a healthy subject's hand in ice-cold water. The bottom left panel shows the variation of LF related to the changes in systolic blood pressure. The average increase of LF is shown in the bar graphs on the right and is reflected in the rise of the LF/HF ratio (∗P < 0.05, n = 15).

In all subjects, Db12 application to cardiovascular variables during the Valsalva manoeuvre showed a significant increase of the LF band on VM and postVM periods (Figure 5, left panels, and Table 7). The same type of computation for RRI values delivered a significant increase of the HF band during the postVM period (Figure 5, right panels, and Table 7).

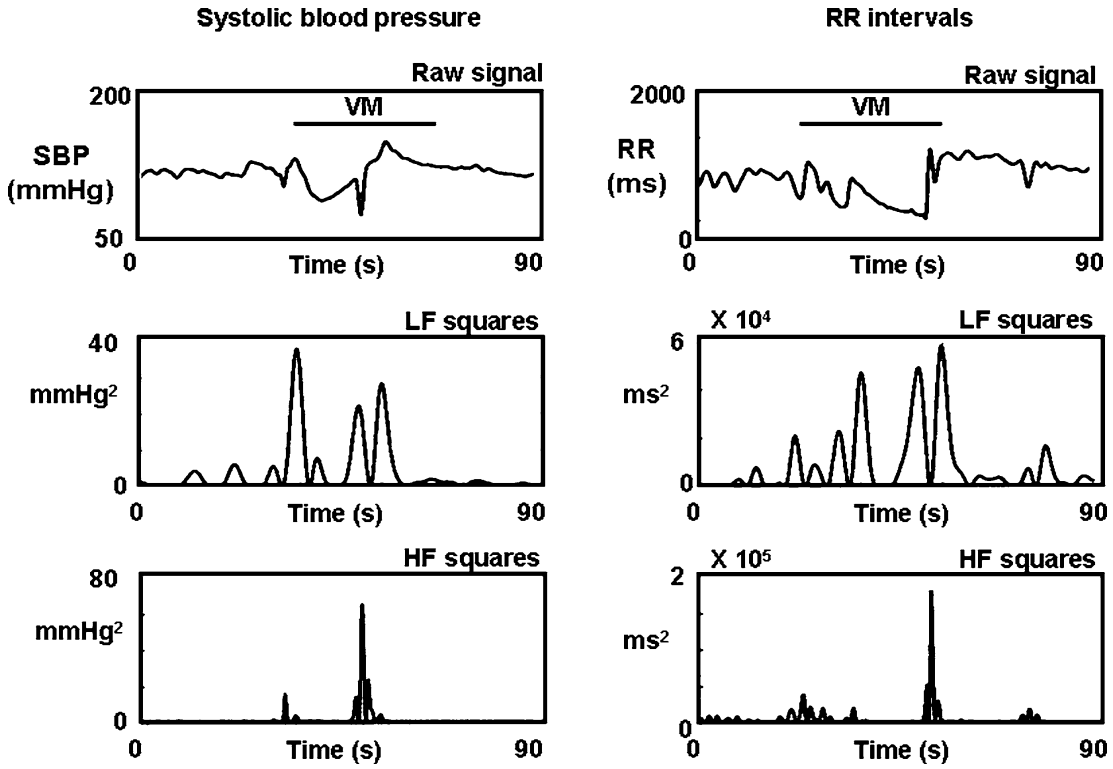


Figure 5. Details of wavelet analysis (Db12) of sBP and RRI signals recorded from a healthy subject during Valsalva manoeuvre.

The underlined changes of sympathetic (seen on LF squares panels) and parasympathetic outflows (seen on HF squares panels).

Table 7. Modifications of LF and HF band values during the Valsalva manoeuvre

	CTR	VM	Post VM
LF_{RRI} (ms^2)	1298 ± 365.7	4596 ± 1191.3*	5270 ± 1363.8*
HF_{RRI} (ms^2)	587 ± 143.1	915 ± 254.8	1619 ± 445.2*
LF_{SBP} ($mmHg^2$)	5 ± 0.9	41 ± 7.9*	22 ± 6.0*

The significant changes in LF bands are shown between control values versus VM and control values versus postVM periods; also shown are the significant changes in the HF frequency band between control and postVM values (for abbreviations see text). Data are expressed as means ± SD. (∗P < 0.05 between control and each individual period, n = 14).

Deep breathing is a predominant parasympathetic activation, and that was clearly reflected in the results of Db12 application. In fact, on wavelets analysis of the RRI signal, the parasympathetic band was shifted to the LF band range of frequencies, with a significant increase of its amplitude (Figure 6, middle and bottom panels).

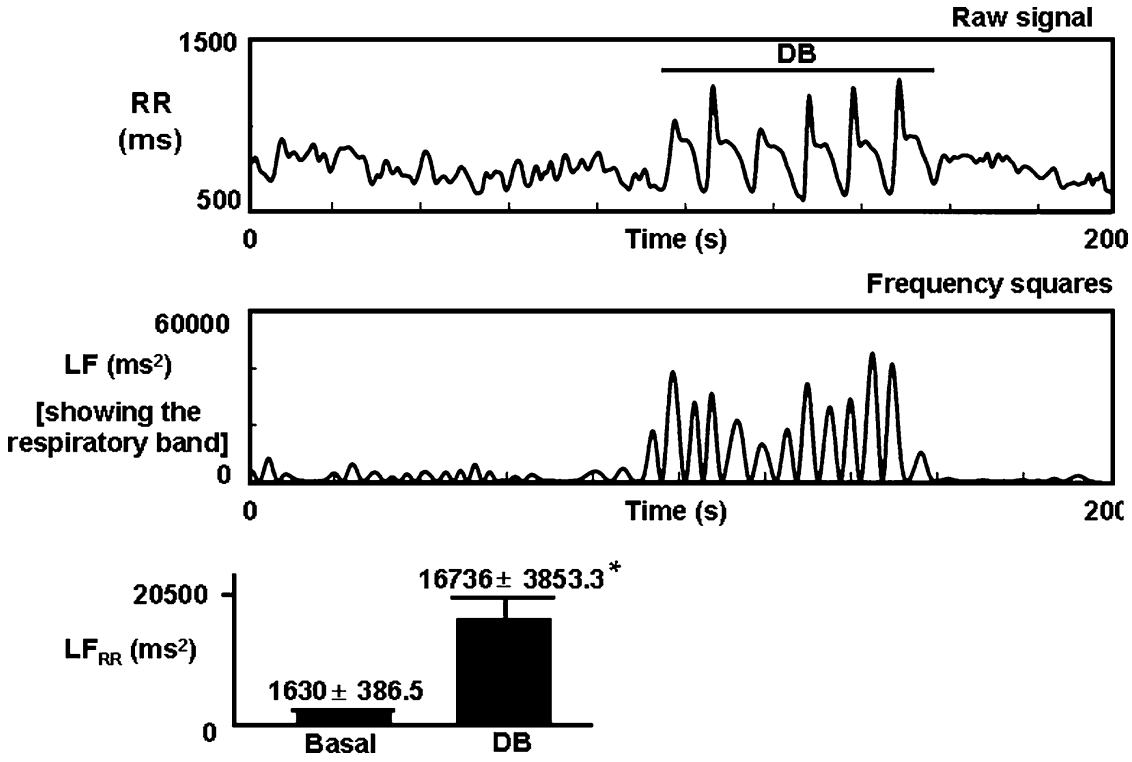


Figure 6. Deep breathing RR intervals analysed by wavelet transform.
Top panel shows RRI variations of a healthy subject during deep metronomic breathing. The middle panel shows wavelet details of RRI signal analysis, illustrating the increase in parasympathetic activity and the shifting of these variations to the LF band. The bottom panel is a bar graph illustrating the significant changes in LF_{RR} values between control and deep breathing values (∗P < 0.05, n = 14).

4.2 Development of a time-frequency tool based on the Hilbert-Huang Transform

As previously described, several approaches to ANS assessment have been developed over the last years. To overcome some of the classical frequency domain algorithms limitations, such as the ones precluding its application to nonstationary and nonlinear signals, we previously described the development of a discrete Wavelet transform algorithm to provide a better description of the time evolution of LF and HF frequency bands' variability. However, being an excellent alternative to classic frequency domain algorithms, wavelets still lack resolution, particularly for the low frequencies band.

A more accurate signal analysis method is the Hilbert Huang Transform (HHT), an adaptively data-driven transform, which holds a higher time and frequency resolution than the short time FFT, and discrete or continuous Wavelet transforms (Peng, Tse, & Chu, 2005; Vigo, Guinjoan, Scaramal, Nicola Siri, & Cardinali, 2005; Ayenu-Prah & Attoh-Okine, 2009; Sosnowski, 2010). By using the HHT, an analysed signal is represented in the time-frequency domain by combining the empirical mode decomposition (EMD) with the Hilbert Transform (HT) (Sosnowski, 2010). In the first step, the EMD, the input signal is decomposed into the so-called intrinsic mode functions (IMF). A finite number of IMFs can be obtained from a real data-series. The IMFs are produced sequentially, with each subsequent IMF being derived from the initial residual signal (i.e., the portion of the initial signal not represented by a previous IMF) (Huang et al., 1998; Huang, 2005b; Chen, Huang, Riemenschneider, & Xu, 2006b; Huang & Wu, 2008).

One limitation found on the traditional HHT is the loss of control at the edges of the interpolation (Wu & Riemenschneider, 2010). The upper and lower envelopes are obtained by cubic spline interpolation during the process of EMD. Since the local maximum and the local minimum cannot be determined at data's starting point and endpoint, severe problems of spline fitting will occur near the ends, where the cubic spline fitting can have large swings, called end effects or end swings. The end swings can eventually propagate and corrupt the entire data span, especially in low-frequency components (Huang et al., 1998). Taking into consideration that this is not a new problem, several solutions have already emerged (Ngo, Apon, & Hoffman, 2010; Wu &

Riemenschneider, 2010; Zheng, Cheng, & Yang, 2014). Arguably, one of the first methods to be published was to apply a time envelope with endpoints going to zero (Wu & Riemenschneider, 2010), much like time windows in the classical spectral analysis methods do. This solution can be acceptable for highly oversampled signals coupled with windows with high roll-off, since the lateral ramps have a low impact on the maximum/minimum and zero equilibrium, however, that does not occur with a tachogram, which is usually poorly sampled. Another common approach is to consider the endpoints to be like mirrors and that there is a ghost reflection of the last maximum and minimum on the other side of the discontinuity on both endpoints. This approach guarantees signal continuity but does not preserve integrity, since too many assumptions are being made on the behaviour of the signal at those endpoints. The two previous approaches fail short of contemplating the non-linear and non-stationary nature of biomedical signals. More complying approaches have also been proposed, being especially interesting the one proposed in (Ngo et al., 2010) where the missing data is filled in by extrapolation inferred through neural networks. This approach, however, has also its shortcomings, namely the added complexity with an inherent increase of computational cost and the high variability of the extrapolation due to the reduced amount of information usually associated with tachograms.

To obviate this problem, in this section we describe the development and mathematical validation of a modified methodology based on the HHT, allowing for smoother upper and lower envelopes, which exhibit IMFs with smaller endpoint oscillations, thus achieving a higher time-frequency resolution and better suitability to signals stemming from nonlinear processes.

I. Methods

A. Empirical Mode Decomposition

The Empirical Mode Decomposition (EMD) is the first mandatory step to work appropriately with non-linear and non-stationary processes. This decomposition is based on the idea that any signal is a sum of smaller and simpler intrinsic modes of

oscillations. Each intrinsic mode represents a linear or non-linear oscillation, which will have a determined number of extremes and zero crosses, guaranteeing that the instantaneous frequency will always be positive (Huang et al., 1998; Huang, 2005b; Huang & Wu, 2008; Feldman, 2009). Besides, each oscillation will be symmetric for a local zero mean. Each of those oscillations can be represented by a function, the Intrinsic Mode Function (IMF).

This function must follow the following rules (Huang et al., 1998):

- In all data, the number of extremes and zero crossings must be equal or differ only by one
- In any point of the data, the envelope defined by the interpolation of all maxima and minima extremes must be zero.

The IMFs are functions with specific frequency and amplitude. The following steps were sequentially implemented to compute all the IMF from an input signal:

1st - all extremes, maxima and minima were identified,

2nd- the upper and lower envelopes were built by a cubic interpolation of all maxima and minima, respectively (Chen et al., 2006b),

3rd - the average between the upper and lower envelope was determined (**10**)

$$h_1 = x(t) - m_1 \qquad (\mathbf{10})$$

4th - Check if h_1 follows the conditions of an IMF.

$$h_{11} = x(t) - m_{11} \qquad (\mathbf{11})$$

If condition (**11**) is not followed, steps 1-4 must be iterated until the first IMF is found. Two stopping criteria associated with this iterative step were implemented: the first one derives from the IMF definition, in which the number of extremes and zero crossings must be equal or differ only by one; the algorithm exits the iteration if this rule is applied

at least four times (Huang et al., 1998). Another exit rule is the Cauchy convergence criterion, which states that the normalised square difference between two iterations should be smaller than a predetermined value (Cauchy, Bradley, & Sandifer, 2009).

$$SD_k = \frac{\sum_{t=0}^{T} | h_{k-1}(t) - k_k(t) |^2}{\sum_{t=0}^{T} h_{k-1}^2} \quad (12)$$

Once SD_k gets smaller than the predefined value, the iteration stops. This condition does not depend on the IMF definition, so it is used together with the first one. Once the first IMF is obtained, it should be shifted from the original signal,

$$r_1 = x(t) - IMF_1 \quad (13)$$

the resultant signal being the residue r_1. The residue may hold more oscillations, so the process of IMF extractions is repeated until the last residue is a monotonic function, from which no more IMF can be sifted.

The signal can be written as the sum of all the obtained IMFs plus the last residue.

$$x(t) = \sum_{j=1}^{n} c_j + r_n \quad (14)$$

The value of the last residue is a constant value or trend line. The first IMF holds the higher frequencies, and the frequencies decrease with the increase of the IMF number, the last IMF being the one with lower frequencies.

B. Hilbert transform

With all IMFs computed, the assessment of instantaneous frequency employing the Hilbert transform is then performed, with two critical benefits: the instantaneous

frequency associated with each IMF is always positive, and each IMF has significance in terms of the underlying phenomena.

The Hilbert transform corresponds to the convolution of the input signal with the kernel,

$$x_H(t) = \mathrm{H}[x(t)] = \frac{1}{\pi \cdot t} * x(t) \qquad (15)$$

Analytically, this corresponds to an improper integral due to the 1/t configuration of the kernel, thus defined by the Cauchy Principal Value,

$$\mathrm{H}[x(t)] = \frac{1}{\pi} PV \int_{-\infty}^{\infty} \frac{x(\tau)}{t-\tau} d\tau \,. \qquad (16)$$

Nonetheless, a more meaningful understanding can be gained from analysing this integral in the frequency domain, which can be achieved by applying the Fourier transform to (**16**).

$$H(f) = \int_{-\infty}^{+\infty} \frac{1}{\pi t} e^{-j2\pi f t} dt = -j\,\mathrm{sgn}(f) = \begin{cases} -j \Leftarrow f > 0 \\ 0 \Leftarrow f = 0 \\ j \Leftarrow f < 0 \end{cases} \qquad (17)$$

From (**17**) it stands out that the Hilbert transform produces a 90º phase lag in the positive frequency range and a 90º lead in the negative frequency range while leaving the amplitude unmodified: the signal remains unchanged, only becoming orthogonal to the input signal. This can be taken with great advantage by defining what is called the analytic signal, *z*(*t*) in (**18**), which corresponds to the time complex-valued function whose real term is the input signal and the imaginary term is the Hilbert transform of the input signal,

$$z(t) = x(t) + jH_T\{x(t)\} = x(t) + jx_H(t) \qquad (18)$$

It is now a simple matter of sorts to derive the instantaneous amplitude of $x(t)$, $a(t)$,

$$a(t) = \sqrt{[x(t)]^2 + [x_H(t)]^2}, \qquad (19)$$

with the instantaneous phase being computed through (**20**)

$$\theta(t) = \arctan\left(\frac{x_H(t)}{x(t)}\right), \qquad (20)$$

and the instantaneous frequency by its time derivative (**21**).

$$f(t) = \frac{\partial\theta(t)}{2\pi\partial t} \qquad (21)$$

A. Modifications to the Hilbert-Huang transform

As previously stated, due to the way the IMFs are sifted from the signal, a question arises regarding how to deal with the endpoint discontinuities in the input signal. The behaviour of the envelopes at the endpoints can create artificial oscillations. These have a remarkably high impact on the output of the HHT, pounding heavily on the physical relevance of the resulting IMFs, on the computational effort required by the HHT and even on the number of resulting IMFs.

We developed a new approach – a modified HHT (mHHT) – during the EMD of the input signal, which consists in identifying the derivatives of the signal at the endpoints, and, by matching these with the derivatives within the signal, we can extrapolate the extremes beyond the endpoints at each side. This, in turn, allows for smoother upper and lower envelopes, which exhibit IMFs with smaller endpoint oscillations.

B. Mathematical validation

The behaviour of HHT and mHHT were first compared using a synthesised stationary

signal (**22**) composed by three sinusoidal functions, each one centred in one of the three bands of interest: VLF, LF and HF,

$$RR(t) = 0.9 + 0.1 \cdot \sin(2 \cdot \pi \cdot 0.3338 \cdot t) + \\ + 0.1 \cdot \cos(2 \cdot \pi \cdot 0.1263 \cdot t + 2) + \\ + 0.05 \cdot \cos(2 \cdot \pi \cdot 0.02 \cdot t + 2) \quad (\mathbf{22})$$

where *t* is the time vector that was built with a length of 120 seconds and a simulated acquisition frequency of two samples per second (Sps). The tachogram was then interpolated at 8 Sps and analysed using both HHT and mHHT, in order to assess their accuracy in extracting the frequency bands of interest.

In a second validation, a stochastic, synthesised signal, more similar to real complex biological signals, was used to assess both HHT and mHHt power to measure dynamic changes of the variability on the frequency bands mentioned above. A complex synthesised signal, modelling the physiological response to an exercise stress test using time-varying autoregressive moving average (ARMA) models (Orini, Bailón, Mainardi, & Laguna, 2012) was selected for this validation. In this model, changes in HF simulate the withdrawal of the parasympathetic modulation in the first 3 minutes after the onset of the manoeuvre (-70% in the first three minutes) and the restoration of baseline values during recovery (+ 50% in 3 minutes). From 3 minutes after the onset of the exercise until the peak stress, HF slightly increases, simulating the effect the stretch of the sinus node (Blain, Meste, & Bermon, 2005; Bailón, Mainardi, Orini, Sörnmo, & Laguna, 2010; Orini, Bailón, et al., 2012).

II. Results

A. Comparison between HHT and mHHT results using the synthesised stationary signal

The IMF's resulting from the decomposition of the synthesised signal (**22**) are shown in Figure 7. The impact of the lack of endpoints modulation on the IMF resulting from HHT analysis can be readily appreciated. The sifting using the traditional HHT algorithm results in an estimated error of 1.8996×10^{-13}. On the other hand, using the mHHT, a lower error of 0.8044×10^{-13} could be estimated, thus suggesting a better performance of our modified HHT in dealing with the endpoints of the signal. Actually, mean estimated frequencies for the IMFs 1, 2 and 3, using the traditional HHT algorithm, were 0.32678 Hz, 0.14012 Hz and 0.041701 Hz, respectively, while mean frequencies for IMF 1, 2 and 3 with endpoint modulation were 0.33332 Hz, 0.12638 Hz and 0.019977 Hz, which represent values closer to the theoretical ones (0.3338, 0.1263 and 0.02, respectively).

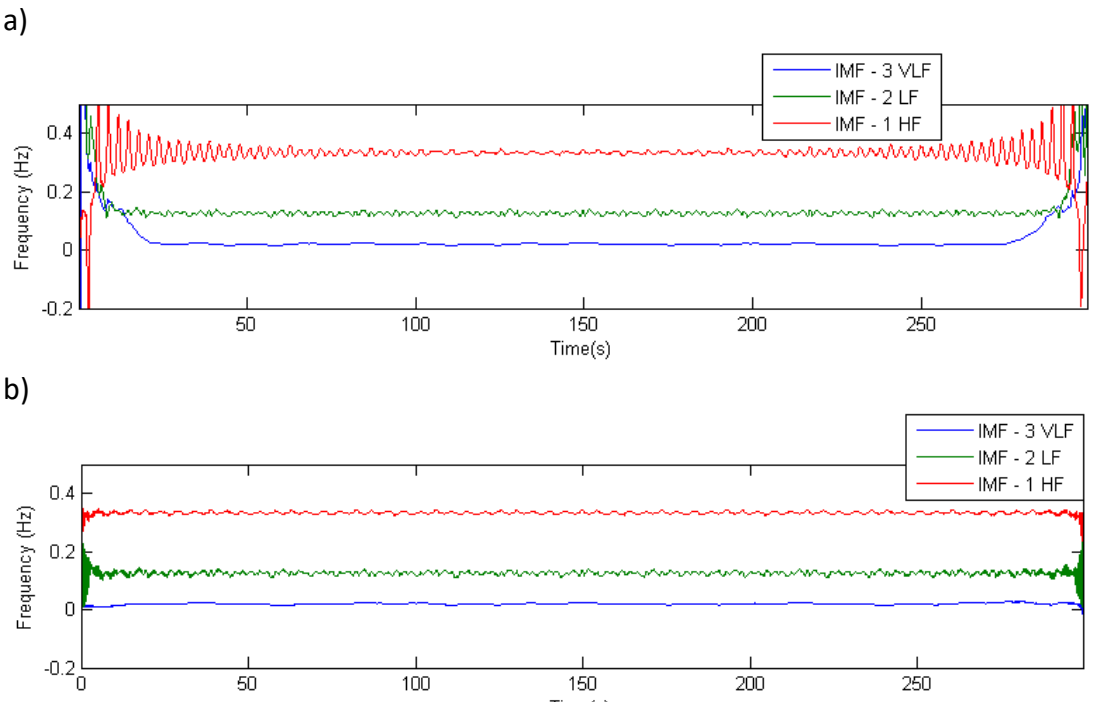


Figure 7. Instantaneous Frequency Plots; Three main IMFs using a) traditional HHT without endpoints control, and b) modified HHT

Power amplitudes along time using HHT and mHHT are shown in Figure 8; mHHT also allows for the computation of a sharper, less noisy, power spectrum, when compared with HHT, as shown in Figure 9.

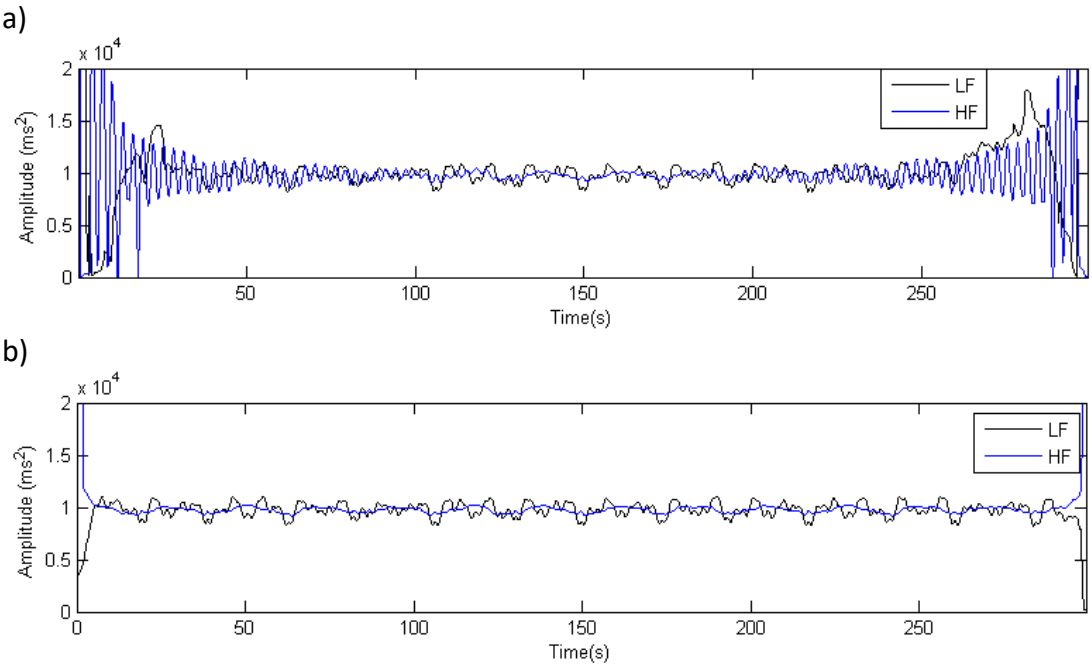


Figure 8. LF and HF Power Amplitudes computed using a) HHT and b) modified HHT.

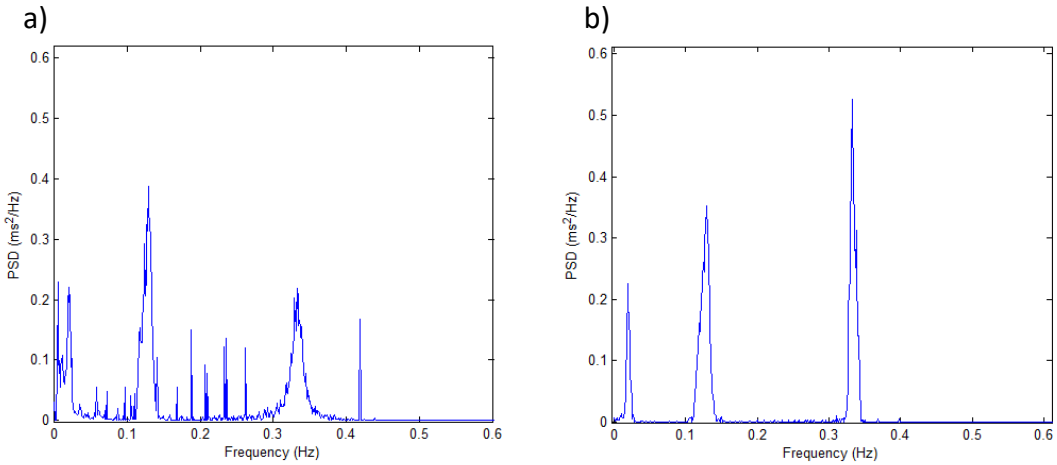


Figure 9. Frequency Power Spectrum (FFT) of the IMFs derived from a) HH, and b) modified HHT

B. Comparison between HHT and mHHT results using the stochastic, synthesised signal

Resorting to the time-varying ARMA model, we synthesised 500 signals with fixed LF and HF frequencies of 0.1 Hz and 0.25Hz, respectively. The model's theoretical HRV amplitude variation is depicted in the top panel of Figure 10. The average LF and HF power plots from 500 modulated stochastic signals using mHHT or HHT are depicted in the middle and lower boxes, respectively.

The mHHT carefully provides an estimation of the HRV when compared to the theoretical variability of this model (Figure 10, top panel), allowing the computation of a cleaner, sharper, power spectrum, when compared with HHT.

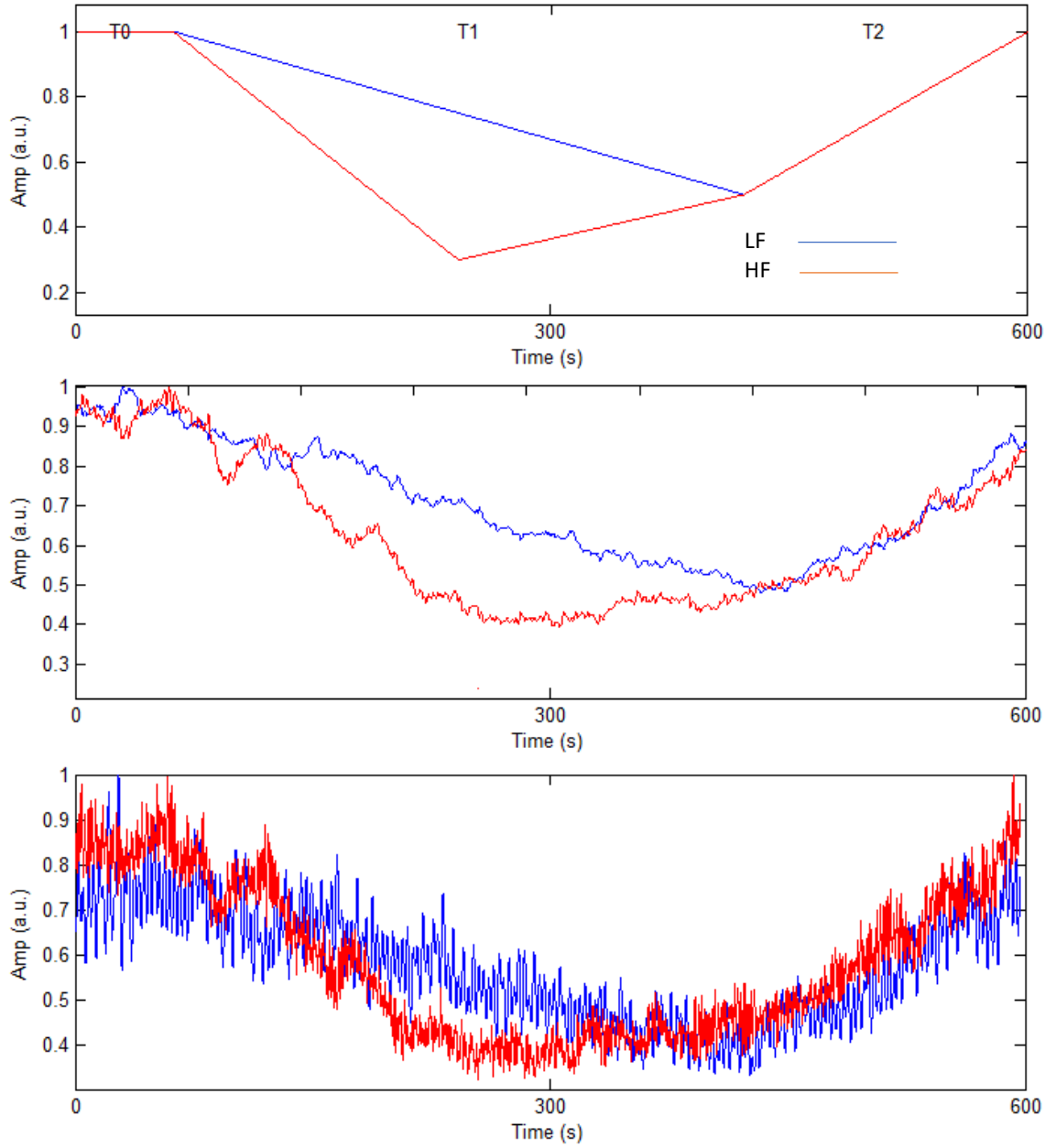


Figure 10. Modelling HRV amplitude during exercise stress testing. Top: model's theoretical HRV amplitude variation; average amplitude of the LF (in blue) and HF (in red) components. Middle: average LF (in blue) and HF (in red) power plots from 500 modulated stochastic signals using mHHT. Bottom: average LF (in blue) and HF (in red) power plots from 500 modulated stochastic signals using HHT.

4.3 Development of a computational tool for the assessment of cardiovascular autonomic nervous system – the FisioSinal Framework

Considering all the fore-mentioned methods used to investigate autonomic function, in the following section we will describe the development of a new integrated computational system, for the assessment of cardiovascular autonomic function.

I. The FisioSinal Framework

The FisioSinal framework was built on MATLAB computational language and organised in a modular approach, incorporating several interconnected modules, grouped according to their functions, as described in the diagram of Figure 11. The data import routine was implemented in order to parse the most common file formats used in our lab, i.e. text raw data files and export formats from LabChart, Holter binary format files, and Taskforce monitor equipment, amongst several other file formats.

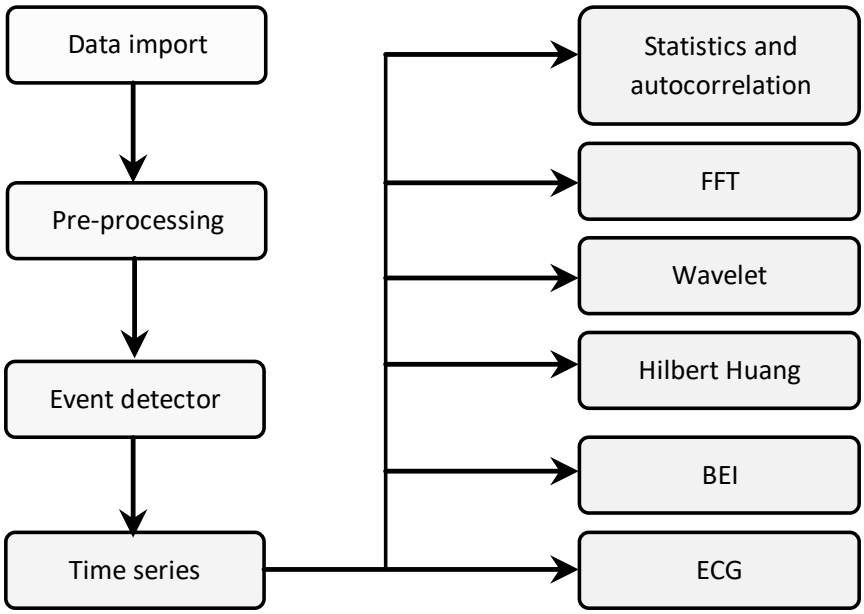


Figure 11. FisioSinal Framework. Diagram of the different modules that compose the FisioSinal framework. *FisioSinal was developed in a modular approach, incorporating several interconnected modules, grouped according to their functions. FFT – Fast Fourier Transform; BEI – Baroreflex effectiveness index; ECG - electrocardiogram*

We will now describe the function and algorithms included in each module.

A. Pre-processing

Biological signals may be disturbed by distinct types of artefacts, namely power-line interference, baseline wander, muscle noise, spikes, and amplitude saturation (Kors & van Herpen, 2010). The goal of the pre-processing module is to remove or minimise the effect of all these interferences on the signal. **Power line** interference is characterised by a periodicity of 50 to 60 Hz. To deal with this power-line interference, we implemented a 3rd order notch filter with a cut-off frequency range of 49Hz and 51Hz in FisioSinal, in order to attenuate frequencies in a narrow frequency band around the interference frequency of 50Hz (Weaver et al., 1968; Kors & van Herpen, 2010). **Baseline wander** originates from changing electrode impedances, such as those caused by respiratory movement (Kors & van Herpen, 2010). The frequency content of baseline wander is typically less than 0.5Hz. A high pass second order "Butterworth" filter, with a cut-off frequency of 0.5Hz, was implemented to reduce the baseline wander. **Muscle noise**, caused by the electrical discharges of skeletal muscles, was reduced using a low-pass filter with a cut-off frequency of 35Hz.

A pre-processed versus a post-processed ECG is shown in Figure 12.

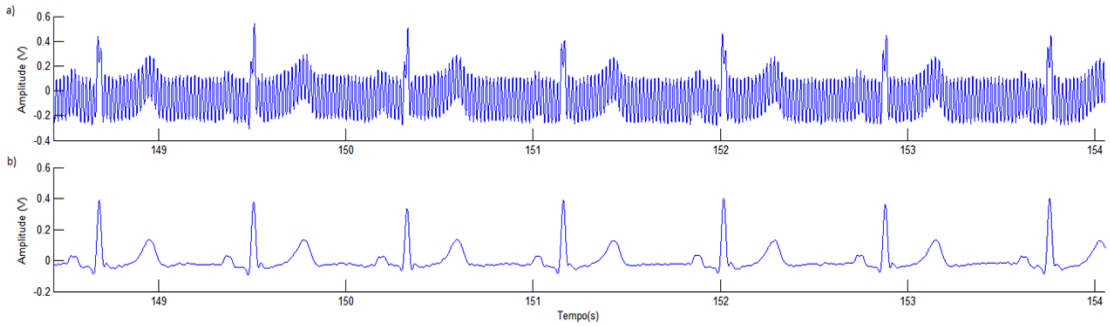


Figure 12. ECG Filters. Application of a 50Hz notch filter, muscular removal filter and baseline wander filter to the raw ECG

B. Waveform detector

The goal of the waveform detector is to determine the fiducial point of the QRS complex, usually the R wave, and to detect the systolic arterial blood pressure in each cardiac cycle. The waveform detector algorithm includes two separate steps: a pre-processing algorithm, followed by a decision rule. In the FisioSinal framework, two sequential methods are employed: a) a wavelet-based QRS detector and b) an adaptative dual-threshold algorithm.

After QRS detection with a Daubechies 12 discrete wavelet transform, a double threshold algorithm (Figure 13) is applied to the processed signal, making the peak detection more robust to noise. The adaptative double threshold algorithm uses a maximum threshold that defines the value above which one event is detected, and a second threshold, which defines the minimum amplitude to be considered in the detection (bellow which is noise). In order to have accurate results, a maximum error of 1 or 2 ms is accepted, which implies that the sampling frequency of the original signals should be between 500 and 1000 Hz.

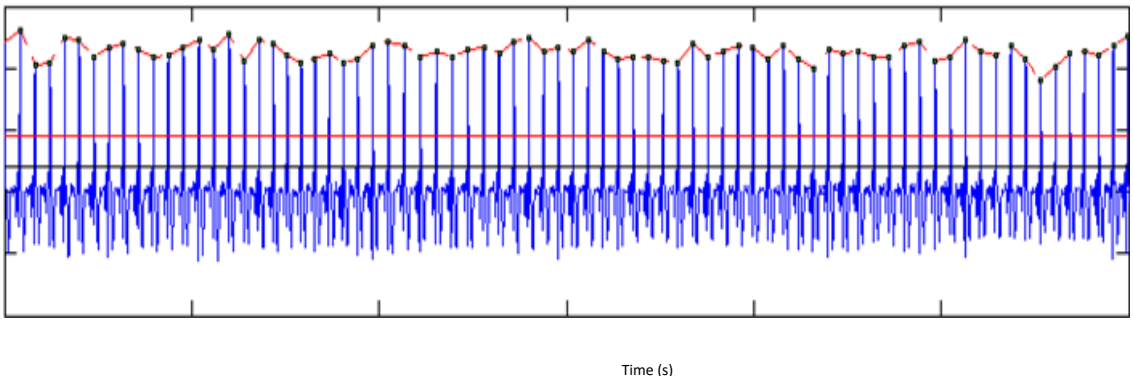


Figure 13. Double threshold event detection algorithm.

In the next step, a waveform typing algorithm is implemented to distinguish between different QRS complex morphologies. If more than one QRS morphology is detected, the classification task is to determine which one is the dominant one and to distinguish them from premature ventricular or supraventricular beats and escape beats (Kors & van Herpen, 2010). In this algorithm, each new complex is compared with one or more of

the already analysed complexes and is then assigned to the group of complexes that are most similar to it, according to two similarity measures (the similarity in shape and in power). If ectopic beats or outliers (including noise) are detected, then they are removed from the time series and replaced by interpolation using a cubic spline (Lippman, Stein, & Lerman, 1994). The parameters of the normal limits in a beat-to-beat interval or systolic pressure can be user-defined. The correctness of this event classification and removal is essential to avoid physiological and pathophysiological misinterpretations.

C. Time and amplitude series construction

After visual inspection and validation of all the automatically detected events, the time series is computed by an 8Hz cubic interpolation over the detected events (Figure 14). This is the last step of the pre-processing of the signal, after which any of the signal analysis can be performed.

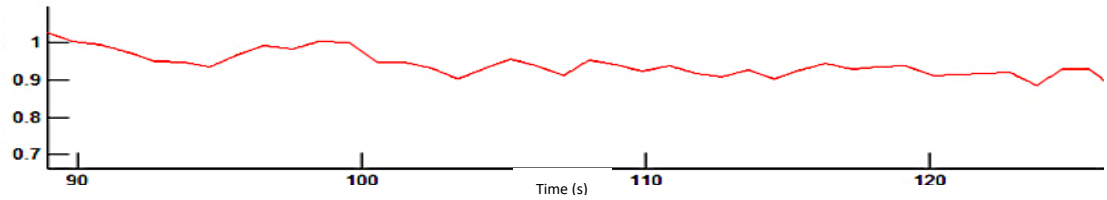


Figure 14. Reconstruction of the time series from events with 8Hz interpolation.

D. Heart Rate Variability module

Six different algorithms were implemented, each under a different domain or function: statistical and geometrical analysis algorithm in the time domain; Fast Fourier transform in the frequency domain; Discrete Wavelet transform, and Hilbert-Huang transforms in the time-frequency domain and, lastly, two baroreflex function assessment algorithms (the sequential method and the Wavelet coherence).

1) Time-domain analysis algorithms

The time-domain analysis methods assess the changes observed in the dependent variable over time and were divided into two categories: *statistical methods and geometric methods*.

- Statistical methods

In this module, indexes are calculated on a beat-to-beat basis. The indices implemented in this module are shown in Table 8.

Table 8. Time Domain Measurements

Variable	Units	Description
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	The standard deviation of the averages of NN intervals in all 5 min segments of the entire recording
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SDSD index	ms	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording
SDSD	ms	The standard deviation of differences between adjacent NN intervals
NN50		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording
pNN50	%	NN50 count divided by the total number of all NN intervals

It is important to note that the comparison between all these variables to bibliographic results, or even between patients, is only valid for similar periods. For short periods of analysis, the shortest period of time suggested is 5 min (Malik et al., 1996).

- Geometric methods

In the group of geometrical methods, the NN intervals are converted into a geometric pattern, such as the distribution of NN intervals or of the differences (delta) between adjacent NN intervals via a histogram or plotted as Poincaré plot.

The following indexes are computed from the histograms:

a) the HRV triangular index (ms), defined as the total number of all NN divided by the maximum height of the histogram of all NN intervals measured on a discrete scale;
b) the triangular interpolation of the histogram NN, which approximates an isosceles triangle on the NN histogram, and measures the base width;
c) the logarithmic index, defined as the ratio of k negative exponential Ae^{-kd} which best approximates the histogram of absolute differences between adjacent NN intervals.

From the Poincaré plot, three parameters are computed: vertical deviation (SD1) and longitudinal deviation (SD2) and SD1/SD2 ratio (Huikuri, 1997). From these parameters, the following indexes are computed: the area of the ellipse by SD1xSD2, the correlation coefficient, the regression coefficient, and the regression equation of the line.

2) Frequency Domain analysis – Fast Fourier Transform module

In the Frequency Domain analysis module, each time series is decomposed using the Fast Fourier Transform into each oscillation composing the full signal. Such a representation results in a power spectrum, displaying the distribution of the amplitude of each oscillation (usually a sine wave) as a function of its frequency. The squared contribution of each frequency is then computed and represents the power of that particular frequency contribution to the total power spectrum (Sosnowski, 2010). As previously described, three frequency bands may be identified, by convention: the high-frequency (HF), the low-frequency (LF) and the very-low-frequency (VLF) bands.

The algorithm used in FisioSinal divides the signal into small segments that can be overlapped by 50% over adjacent segments (the degree of overlap may be personalised). The implemented windows of analysis include the rectangular, Barlet, Parzen, Hanning, Hamming and Welch windows (Welch, 1967; Harris, 1978; Hayes, 1996; Heinzel, Rüdiger, & Schilling, 2002). The power spectrum density is then calculated by measuring the area under the curve of the respective bands.

The powers of VLF, LF and HF are then measured in absolute (ms^2) and in normalised units (nu), the latter representing the relative portion of the power band on the total power minus the power of VLF band (Malliani, Pagani, Lombardi, & Cerutti, 1991; Malliani, 2000).

3) Discrete Wavelet transform and Hilbert-Huang Transform module

The discrete wavelet transform and the Hilbert-Huang Transform have been previously extensively revised in this chapter. Please refer to the specific sections for more details.

4) Baroreflex Assessment Module

Baroreflex sensitivity was computed using the sequence method (Parati, DiRienzo, & Mancia, 2000; Di Rienzo, Parati, et al., 2001). The sequence method is based on the spontaneous presence of concurrent R-R interval and sBP changes (increase or decrease) over at least three consecutive heartbeats (sequence)(Di Rienzo, Parati, et al., 2001b; Sosnowski, 2010). In brief, this estimation was based on the analysis of beat-to-beat series of sBP scanned to identify ramps of 3 or more consecutive heartbeats with a progressive increase ("up-ramp") or decrease ("down-ramp") of at least 1 mmHg, regardless of the possible occurrence of concomitant RR interval changes.

The algorithm identifies spontaneous sequences, defined as sBP ramps, followed by concomitant and concordant RR intervals variations of 5 milliseconds coupled with 0-, 1-, and 2-beat lags, with each sequence being included only once. For each sequence, the slope of the linear interrelationship between sBP and the following RR intervals values is calculated and considered reliable when the correlation coefficient (r) is higher than 0.80.

For each period of analysis, the baroreflex effectiveness index (BEI) is defined as the ratio between the total number of baroreflex sequences detected and the total number of sBP ramp-like changes in blood pressure, regardless of whether the latter is followed

by a change in RR-interval or not (Di Rienzo, Parati, et al., 2001b). The higher the effectiveness index value, the more sBP ramp changes are followed by a change in RR-interval.

$$BEI(\%) = \frac{num\ events\ BRS}{num\ ramps\ BP} \cdot 100 \quad \textbf{(23)}$$

5) Cross-Spectral Wavelet Coherence and Phase

The coherence is a complex function of the frequency and estimates a degree of linear correlation between two signals. High coherence suggests the capability of one time series to predict the following one (Sosnowski, 2010). Availability of the cross-spectrum also allows for the calculation of the phase relationship in the range of -180º and +180º, with the phase relationship being represented as the phase spectrum.

In the FisioSinal framework, Cross Spectral Wavelet Coherence analysis was developed based on the seminal works of Torrence and Compo (Torrence & Compo, 1998) and Grinsted et al. (Grinsted, Moore, & Jevrejeva, 2004). In brief (Yang et al., 2008; Laranjo, Tavares, Oliveira, & Rocha, 2014), a complex wavelet transform (Morlet wavelet) is used for feature extraction from cardiovascular signals analysed. Continuous wavelet transform (CWT) scalograms are calculated for the sBP and the heart period variables. From the two CWTs, a Cross Wavelet Transform (CrWT) is constructed, exposing the common power and relative phase in time-frequency space. Next, the Wavelet Coherence (WTC) between the two CWT, is computed. The coherence spectrum, presenting values between 0 and 1, is a measure of the correlation between the variations of the two signals for a given frequency. The phase spectrum shows, at each frequency, the phase difference (lead or lag) between the signals, from where the lag period (in seconds) between the two time series is computed.

E. Graphical user interface

All of the above modules and functions were implemented into one system – FisioSinal,

which has an interface that allows intuitive control of all analysis functions and immediate assessment of the results (Figure 15).

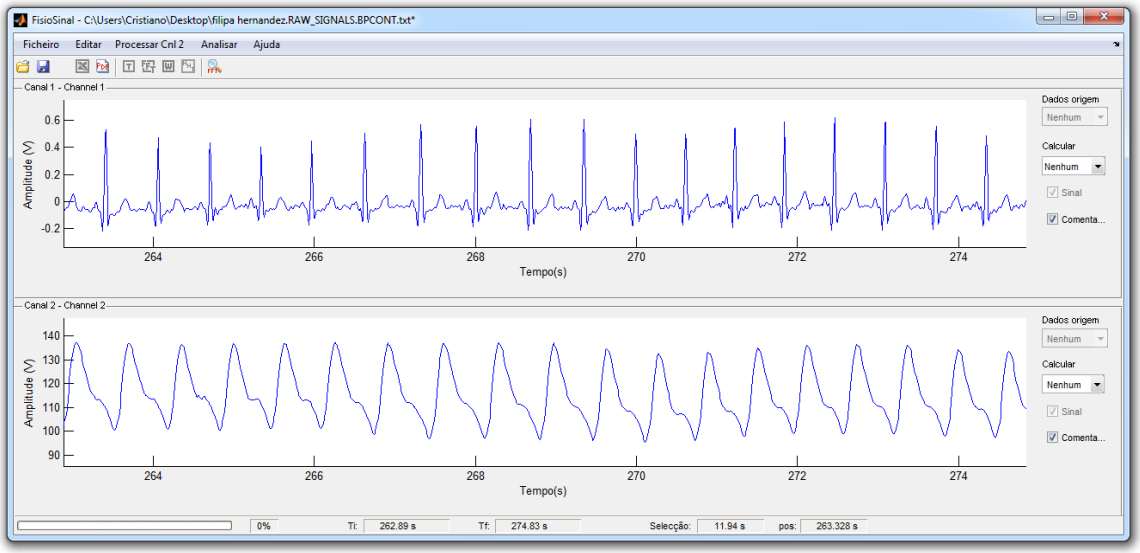


Figure 15. FisioSinal Main Window

This interface allows for the visualisation of two simultaneous signals, synchronous in time. All program functions and analysis are controlled from this window. It is possible to tighten or expand the time scale for a more precise visualisation and choice of the signal segment to be analysed.

F. Clinical Application

In order to illustrate the mode of function of the FisioSinal, we will present two signals acquired using a Taskforce Monitor (TFM) model 3040i (Cnsystems, Austria) during a head-up tilt from a healthy 36-years old male and a 45-year old paroxysmal atrial fibrillation female patient in sinus rhythm. The ECG was acquired at a sampling frequency of 1000 Hz with an accuracy of +/-5V, which is synchronised with the continuous measurement of blood pressure, acquired with a sampling frequency of 100 Hz. This equipment uses an analogue-digital processor to convert the 3-channel ECG and pressure to digital format. The raw data were pre-processed, as previously described.

The analysis starts 2 minutes before the head-up tilt and ends 2 minutes after the end of the manoeuvre, for both individuals. The systograms from both individuals are presented in Figure 16.

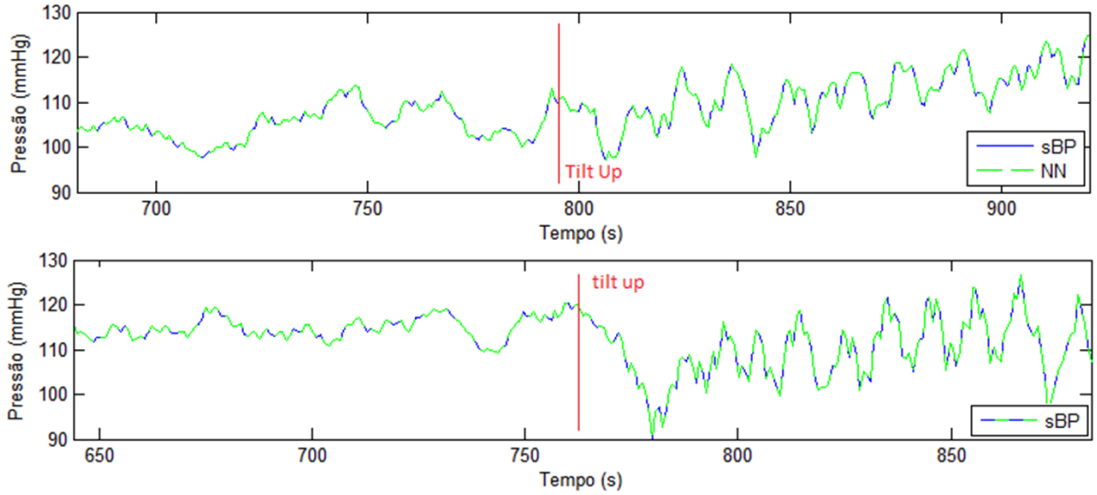


Figure 16. Systograms. From a healthy individual (top panel) and a paroxysmal atrial fibrillation patient (bottom panel). The beginning of the head-up tilt test is marked by the red vertical line.

Time-domain analysis

The results obtained from the time domain analysis methods are presented in Table 9.

Table 9. Time Domain Results for Basal and Head-up tilt

	Healthy Individual		Paroxysmal Atrial Fibrillation Patient	
	Basal	Tilt-up	Basal	Tilt-up
Normals RR	193	204	135	149
Ectopic	0	0	0	0
Max (mmHg)	113.7	123.5	120.5	126.5
Min (mmHg)	97.6	97.1	109.2	90.2
Mean (mmHg)	104.9	111.2	115.0	109.8
Median (mmHg)	104.6	111.7	114.8	110.3
SD	0.004	0.006	2.509	6.893
RMSSD	0.001	0.002	1.116	4.101

The Poincaré plots are presented in figure 17.

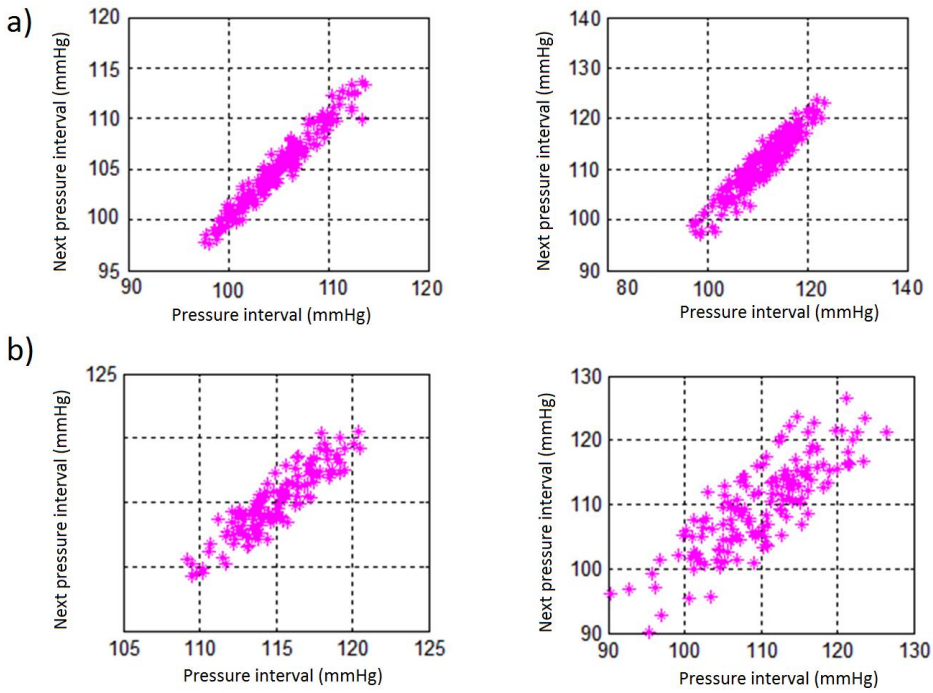


Figure 17. Poincaré plots of basal (left panel) and during tilt-up (right panel) for a healthy individual (a) and a paroxysmal atrial fibrillation patient (b).

For the healthy individual, in the basal supine position, the dispersion values for the SD1, SD2 were 1.08 and 4.94, respectively, increasing during the manoeuvre to 12.50 and 7.43.

On the other hand, the paroxysmal atrial fibrillation patient presented significantly higher values, both at the basal supine position (SD1 and SD2 are 5.17, 3525.14) and during the manoeuvre (74.52, 9701.92, respectively).

The paroxysmal atrial fibrillation patient has a more disperse Poincare plot, indicating higher dispersion values during tilt.

Frequency-domain analysis

The FFT power spectrum is presented in Figure 18. Three well-defined frequency bands (VLF, LF and HF) can be identified. The paroxysmal atrial fibrillation patient presented higher LF power both in the supine and orthostatic position when compared to the healthy individual. A substantial increase in the spectral peak in the LF range after head-up tilt could be demonstrated, translating an activation of the sympathetic branch of

the ANS. This result is expected for this type of manoeuvre.

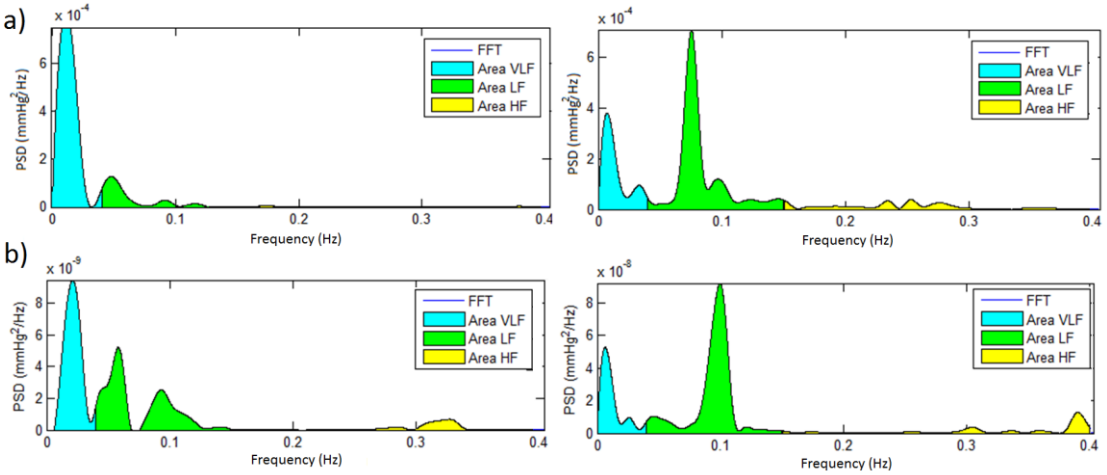
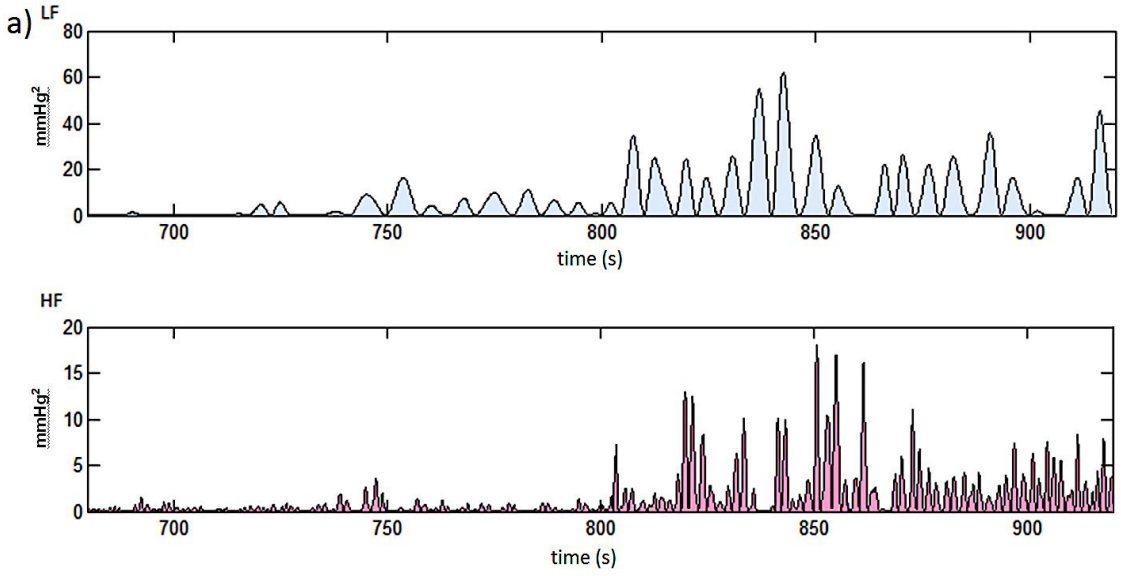


Figure 18. FFT spectrum. Two minutes of basal (left panels) and two minutes after tilt (right panels) for a healthy individual (a) and a paroxysmal atrial fibrillation patient (b).

Time-Frequency-domain analysis

The spectra produced by the discrete wavelet transform is shown in Figure 19.



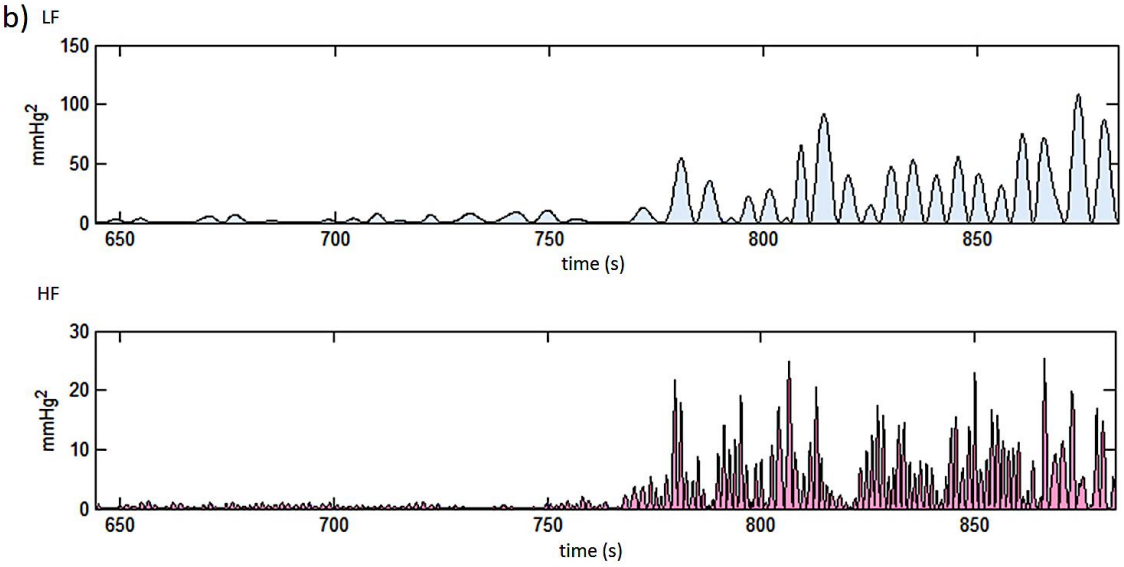


Figure 19. Wavelet Transform Spectrum a) healthy individual, b) paroxysmal atrial fibrillation patient.

The graphics of the LF and HF spectra show an evident change between the baseline and the period of manoeuvre, which starts at 760 seconds. It is possible to see a substantial increase in the LF and HF powers when compared with baseline, confirming the assumptions expressed by FFT analysis. Modified Hilbert-Huang analysis was then performed, and the power spectrum of the LF and HF bands are presented in Figure 20.

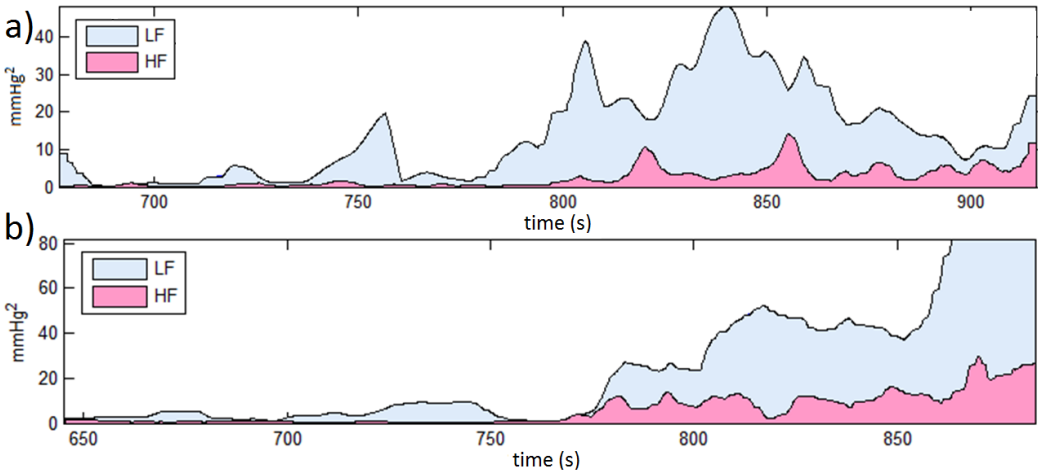


Figure 20. Hilbert Huang Transform Spectrum. a) healthy individual, b) paroxysmal atrial fibrillation patient.

The modified Hilbert-Huang transform spectrum was similar to the one produced by the discrete wavelets transform, with a higher resolution as an advantage. A distinct pattern

of response could be seen in the paroxysmal atrial fibrillation patient, with a delayed but stronger and longer increase on the LF variability, while for the healthy subject a faster, sharper and more efficient adaptation to the assumption of orthostatic position was found.

4.4 Discussion

The main goal of this chapter was the development and validation of two mathematical algorithms for the quantification of autonomic function through the evaluation of heart and blood pressure variability along time. A modification to the Hilbert-Huang transform is also presented, resulting in more accurate and sharp results, by better managing the signal distortion caused by endpoint handling. The validation of these tools was done using three classical provocative autonomic manoeuvres - cold pressor test, Valsalva manoeuvre and the deep breathing - whose results are usually analysed with the Ewing protocol and through the analysis of two synthesised signals. The validation of both the discrete wavelet transform Db12, and the modified Hilbert-Huang transform as mathematical tools for dynamic autonomic analysis led to the development of a new computational framework for autonomic evaluation comprising tools on the time, frequency and time scale domains, the FisioSinal.

On the validation of wavelets Db12 for autonomic evaluation

Due to the need of a tool able to dynamically analyse the rapid changes on the autonomic outflow, and to overcome the previously described limitations of FFT, we have developed a new mathematical wavelet-based tool for autonomic evaluation on time-frequency domain analysis, in the present chapter.

Wavelet methodologies have been fine-tuned over the years for time-dependent spectral analysis (Mallat, 2009). Presently, it is possible to choose from a wide range of convolution kernels, according to the signal's specific features. In fact, the discrete wavelet transform, which was developed for geological purposes, has been shown by us (Ducla-Soares et al., 2007) and others (Wiklund et al., 2002; van den Berg et al., 2005;

Moriguchi et al., 2006; Urbančič-Rovan et al., 2006) to be applicable to biological signals, in particular cardiovascular signals.

In the present study, by choosing the limits of each detail, we studied the contribution of two sets of frequencies, LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) (Malik et al., 1996), to rapid and acute changes in heart period (using RRI) and sBP evoked by each of the autonomic tests described.

The cold pressor test is used to activate pain and temperature fibres by the immersion of one hand and arm in ice-cold water (0–4°C) for 40–180 s (Hilz & Dütsch, 2006). The sympathetic nervous system is activated through the spinothalamic tract, inducing an increase in blood pressure and heart rate, but sBP is a more reliable measurement (Fasano et al., 1996; Cevese, Gulli, Polati, Gottin, & Grasso, 2001). During this test, we measured sBP acute and rapid changes, which were compared between the resting period and the maximum response during the immersion of the subject's hand in iced water. The direct relation between sympathetic outflow and sBP rise is also well noticed through wavelet computation of sBP signal, which shows an increase of LF band induced by cold temperatures, without significant changes in HF. These data are in line with results of various authors using other mathematical methods, which indicate that in terms of autonomic influences on cardiovascular variables such as blood pressure and heart rate, the sympathetic nervous system has a more direct influence on blood pressure, whereas the parasympathetic nervous system predominantly affects heart rate. Also, the focus on blood pressure analysis during CPT is in accordance with the Ewing protocol results, which classically only evaluate blood pressure when the cold stimulus is applied, as justified by the reasons stated above.

In the Valsalva manoeuvre, intrathoracic pressure increases abruptly owing to forced expiration against a closed glottis, causing an increase in heart rate mediated by the carotid baroreceptor reflex (Levin, 1966; Ekholm & Erkkola, 1996). In fact, inputs from the arterial baroreceptors play the leading role in the compensatory reflex response to the increase in intrathoracic pressure (Sharpey-Schafer, 1955; Eckberg, 1980a, 1980b; Looga, 2005). Cardiovascular responses elicited during the Valsalva manoeuvre can be

divided into four main phases; two of them reflex in nature. During *phase I* of VM, a small increase in pressure and a decrease in HR were observed and in *phase II*, the first reflex phase, BP decrease unloads the baroreceptors and evokes an increase of sympathetic outflow. This is shown in our analysis as an increase in LF in sBP and RRI powers. After the release of the respiratory strain in *phase III*, there is a BP decrease and an HR increase. *Phase IV* of VM, the second reflex phase, is characterised by a BP rise that evokes baroreceptor-mediated bradycardia, which is also well shown in HF_{RRI}.

The most common manoeuvre to evaluate parasympathetic activation is the deep breathing at six breaths min^{-1}. At this respiratory rate, the processing of the RRI signal shows a shift of parasympathetic-mediated changes into the LF range, with its significant increase in the amplitude (Hohnloser & Klingenheben, 1998). Accordingly, the parasympathetic outflow should be analysed on the LF range of frequencies, since this shifting is due to the fact that the respiratory rate is coincidental with the LF range of frequencies. This analysis is only valid in conditions in which the frequency and breathing volume are carefully controlled (Hartikainen, Tahvanainen, & Kuusela, 1998).

With the present results, we also intended to relate the clinical evaluation of autonomic function by the standard autonomic tests performed routinely in an autonomic laboratory, to the autonomic responses elicited by each manoeuvre analysed in a more detailed way by wavelets. In fact, in clinical practice, it is accepted that heart rate changes during the Valsalva manoeuvre or deep breathing test reflect parasympathetic function, while the increase in arterial blood pressure during the cold pressor test reflects sympathetic outflow (Ewing & Clarke, 1982; Ewing, Martyn, Young, & Clarke, 1985). Thus, our results demonstrate the relative change in autonomic activity, which is responsible for the cardiovascular changes in all four autonomic tests.

In conclusion, wavelet analysis provides new opportunities for analysing acute and transient changes in cardiovascular (and autonomic function) variables within periods of less than 5 minutes, which are not accessible to FFT. Also, by overcoming the FFT requirement of a long and stationary signal recording, the analysis of variability of biological signals in the time-frequency domain using wavelets is a useful tool to

evaluate autonomic function in routine medical practice.

On the application of Hilbert Huang transform to autonomic evaluation

The Hilbert-Huang transform is widely used in various fields dealing with nonlinear and nonstationary signals (Huang & Shen, 2014). To overcome the drawbacks of the HHT (namely, the way endpoints are handled during the process of Empirical Mode Decomposition), a modified HHT has been presented in this work. In our algorithm, we infer how the interpolation equation should behave, through the similar derivative of the endpoints.

The modified HHT algorithm was compared against the application of the HHT to the same synthesised signals. Simulation results show that the modified HHT can effectively suppress end-effects, resulting in a cleaner, higher resolution IMFs and in better discrimination of the variability encoded in the LF and HF frequency bands of both synthesised signals, which indicates that the modified HHT method is more accurate than the original one.

By better adapting to non-linear signals, like biological signals, the modified HHT has a better time-frequency resolution when compared with the traditional HHT, but also with the results derived from the discrete wavelet transform. However, further work using this method applied to large groups of individuals – healthy subjects and patients – should be performed in order to establish normality data for both physiological and pathophysiological conditions.

On the development of a platform for autonomic evaluation to be used in physiology and medicine

The analysis of HRV is nowadays a standard method for non-invasive evaluation of ANS integrity. In this work, we developed an integrated and modular system - FisioSinal - capable of clinical and laboratory evaluation of the behaviour of the ANS using cardiovascular signals in humans and animal models.

The computational tools that were included seek to cover the currently most validated methodologies: *stochastic analysis, descriptive statistics, auto-regression, fast Fourier transform, discrete Wavelets transform and baroreflex sensitivity index*. Due to its limitations, we developed and validated a new analytical tool based on HHT. FisioSinal was validated and tested by analysing a synthesised signal. The clinical applicability was demonstrated through an analysis of the cardiovascular signals of a healthy individual and a patient with paroxysmal atrial fibrillation, undergoing an autonomic provocation manoeuvre.

This work achieved the aim of building a more natural and more versatile analysis tool for ANS assessment, through HR and BP variability. In this sense, we brought together in one framework, several algorithms and analysis methodologies, allowing a fast learning curve for new users with shorter computation time. In this work, we also developed a new algorithm of analysis with the introduction of the HHT. This new module has been optimised for autonomic analysis and should be useful in future work since it goes beyond some of the limitations of the traditional methods of analysis.

Also, being a modular tool, the addition of new mathematical algorithms as they will be required, is much easier. Presently, the FisioSinal is a prototype in use at the Cardiovascular Autonomic Function Laboratory and has been proved by users as an improvement in data analysis leading to an increase in diagnostic accuracy and a closer and personalised follow-up of our patients.

CHAPTER 5

CHAPTER 5

AN INSIGHT INTO THE AUTONOMIC AND HAEMODYNAMIC MECHANISMS UNDERLYING REFLEX SYNCOPE

Exploring the hypothesis under study…

Various studies on the pathophysiology of reflex syncope have been performed to understand the underlying mechanisms that ultimately end up in a transient loss of consciousness. However, so far, doubts still arise about the role of the autonomic nervous system on the initiation, maintenance and termination of the syncopal event. Along with several works, attention has been given to the efferent pathways of the autonomic reflex arc and the changes on autonomic cardiovascular reflexes, but results still vary among authors. There are various reasons for this lack of scientific agreement: the cohort of patients and their inclusion and exclusion criteria, the distinct testing procedures and the various methodologies of data analysis. We also believe that mechanistic knowledge is crucial for the development of a coherent representation of the disease, being a way of retaining knowledge over time and maintaining diagnostic accuracy. So, attempts should be made to develop straightforward connections between mechanistic knowledge and clinical practice.

Thus, in accordance, as a working hypothesis, we establish that

mechanisms of reflex syncope underline autonomic and haemodynamic responses which evaluation by dynamic signal processing tools will lead to the improvement of clinical decision making and of the patient's quality of life.

I. Introduction

Vasovagal syncope, despite its frequent occurrence, is still an enigma regarding what triggers the downward spiral of events that lead to hypotension, bradycardia and loss of consciousness. Several studies have attempted to understand the pathophysiology of reflex syncope, but due to its multiple factors, there are still considerable doubts about the underlying mechanisms that terminate in a transient loss of consciousness. The evidence in the literature is conflicting about the role of both autonomic nervous system (ANS) limbs in the genesis of vasovagal syncope. Similarly, the role of an alteration in the arterial baroreceptor reflex, predisposing subjects to vasovagal syncope, has been suggested or denied by different studies. The baroreceptor response to changes in blood pressure (BP) is characterised by its gain (or sensitivity) and by the time delay (phase or latency) of its response (Thrasher, 2004; Gulli, Cooper, Claydon, & Hainsworth, 2005). The gain of the baroreflex response has been widely investigated using a variety of methods (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; Gulli, Claydon, Cooper, & Hainsworth, 2005). Much less attention has been given to the latency of the baroreflex (Gulli, Claydon, et al., 2005; Alvarez-Ramirez, Rodriguez, & Echeverría, 2009), which may underlie diagnostic and prognostic value by itself or in combination with other clinical tools and markers.

Furthermore, while studies in the adult population with vasovagal syncope are abounding, only a few studies (with conflicting results) have investigated the vasovagal syncope mechanisms in paediatric patients. Indeed, some authors have reported that children with reflex syncope had alterations in baseline autonomic balance, whereas others did not find any modification on basal autonomic output (Stewart, Erb, & Sorbera, 1996; Massin, Henrard, & Gerard, 2000; Alehan, Ayabakan, & Özer, 2002; Kochiadakis et al., 2004; Nowak, Ocon, Taneja, Medow, & Stewart, 2009). Although both types of studies agree that the ANS appears to play an essential role in the pathogenesis of reflex syncope, many questions remain unanswered regarding the precise aetiology and pathophysiological mechanisms of the autonomic response to orthostatic stress in paediatric patients.

In the present section, our main aim was to evaluate the haemodynamic, autonomic

cardiovascular and baroreflex responses in reflex syncope patients (both adults and children), submitted to a head-up tilt test (HUT). In particular, it was our goal to a) better clarify, using dynamic signal processing tools, the background of the autonomic and haemodynamic responses elicited by HUT in patients with a history of recurrent vasovagal syncope; b) to characterize baroreflex function in terms of gain and latency, and finally, c) to identify surrogate markers that would allow us to predict the response to HUT in patients with vasovagal syncope.

II. Methods

A. Inclusion and Exclusion Criteria

Between January 2012 and June 2019, two groups of patients with reflex syncope were enrolled in this study: an adult (age ≥ 18-year-olds) and a paediatric (age < 18 years old) patient group. For the adult population, a group of healthy individuals age and sex-matched (control group) were enrolled in this study. All patients met the following inclusion criteria: 1) at least two syncopal episodes within the last six months or one syncope and three pre-syncopal episodes/year (defined as a transient alteration of consciousness, without complete loss (Peinado, 2006); 2) absence of known structural and/or electrical heart disease; 3) absence of other evident aetiologies for syncope; and, 4) absence of criteria for orthostatic hypotension, defined as a sustained reduction of systolic blood pressure of at least 20 mm Hg or diastolic blood pressure of 10 mm Hg within 3 minutes of standing or head-up tilt to at least 60° on a tilt table (Freeman et al., 2011; Cheshire, 2017). Subjects with concomitant chronic or acute disease, namely history of cardiovascular disease, carotid sinus syndrome or any underlying situation that might affect the ANS function, smokers and those undertaking any chronic medication were excluded from the study. Physical examination, electrocardiogram, and echocardiogram were normal for both patients and control group subjects. The study, following the Declaration of Helsinki and the Oviedo Convention, was approved by the *Centro Hospitalar e Universitário de Lisboa Central* and the Faculty of Medicine of the University of Lisbon's Ethics Committee, being performed under written, informed consent from all participants.

B. Experimental Protocol

Head-up tilt table test (HUT) was performed in a dedicated laboratory, in a quiet and dimly lit environment with controlled temperature and humidity, during the morning period, after a light breakfast, without ingestion of caffeine, chocolate or other xanthines. No intravenous lines were used. Subjects were placed on a tilt table and, after a resting period of 10 minutes in the supine position, were tilted-up to a level of 70º, for 20 minutes or until symptoms occurrence. Subjects were instructed to breathe regularly and were asked not to speak, except for comments about symptoms or to mention discomfort. Breathing rate and amplitude were controlled through a respiratory belt. As soon as symptoms (syncope or pre-syncope) developed during the adaptation period, the table was lowered to the horizontal position, and the test was concluded. Following the return to the supine position, a second resting period of 5 minutes was allowed to elapse, so as to assure a stable condition. A positive response to HUT was defined as a sudden development of syncope or pre-syncope associated with hypotension, bradycardia, or both, while a negative test was determined as the absence of hypotension, bradycardia, syncope, or pre-syncope. Positive responses types were in accordance with the VAsovagal Syncope International Study (VASIS) classification: a vasodepressor response (type 3), defined as a marked fall in systolic blood pressure of, at least, 30% or below 70 mmHg associated with the reproduction of symptoms; a cardioinhibitory response, defined as an abrupt fall in heart rate, with or without asystole (types 2B and 2A) and a mixed response (type 1) characterized by both blood pressure and heart rate decrease. The control group (healthy individuals matching the age, body mass index and sex) was also subjected to the same experimental protocol.

C. Data Acquisition

During the HUT test, patients were continuously monitored using a Task Force Monitor (Model 3040i; CNSystems, Graz, Austria)(Fortin et al., 2006). Heart rate and R-R interval (RRI) were obtained from the ECG. The ECG was acquired at 1 kHz with an accuracy of ±5 μV. Using the vascular unloading technique (Imholz, Wieling, Van Montfrans, & Wesseling, 1998), continuous blood pressure (BP) was recorded from the second or middle finger of the right hand and calibrated against the oscillometric measurement of

arterial blood pressure in the left arm. Impedance cardiography, a method in which a small electrical potential is applied between three-band electrodes on the neck and upper abdomen, was used to obtain a continuous recording of the temporal derivate of the transthoracic impedance (dZ/dt)(Denniston et al., 1976).

D. Data Analysis

All raw, unfiltered, data were exported to a dedicated workstation and analysis was performed using an in-house computational interface - FisioSinal. Beat-to-beat stroke volume and end-diastolic volume index were calculated from the impedance signal (Fortin et al., 2006). Cardiac output was calculated from heart rate and stroke volume. The total peripheral resistance index was calculated from mean arterial BP and impedance cardiography data (Fortin et al., 2006).

The raw signal was filtered in order to remove movement and electrical noise. The signal was further filtered using a polynomial trend removal algorithm and a bandpass filter to isolate the periodicities of interest and minimise the effects of non-stationarities. A continuous visual inspection was subsequently performed in order to ensure that only data that does not contain any abnormal beats were extracted for further analysis. A peak-to-peak routine was implemented to detect systolic blood pressure (sBP) peaks and the fiducial points of the R waves of each ECG complex, so as to construct a time curve of BP (systogram) and of HR (tachogram). The RRI and sBP were interpolated by a cubic spline and resampled (resampling period =0.193 s). The reconstructed signals were used to calculate heart rate (HR) and sBP variability and baroreflex sensitivity. In addition to the control group, patients were divided into the following two groups, according to their response to HUT: tilt-positive patients [fainters] and, tilt-negative patients [non-fainters]. The analysis of each cardiovascular parameter from the three groups was carried out in six consecutive moments, with a 5 minutes length (Malik et al., 1996; Berntson et al., 1997): 1) baseline period: the 5 minutes of recording immediately before tilting; 2) the 5 minutes of recording immediately after tilting-up; 3) 5 minutes segment immediately after the second period; 4) the 5 minutes segment immediately after the third period; 5) at the end of tilt, corresponding to the last 5 minutes prior to development of symptoms; or, the last 5 minutes on tilting-up for tilt-

negative patients. The last segment comprises the first 5 minutes of recovery on supine position starting 15 seconds after tilt-down.

i) Heart rate and blood pressure variability

Heart rate and systolic blood pressure variability were assessed through the modified Hilbert-Huang transform (please refer to *Chapter 4* for more details). Briefly, this method first decomposes the signal into a finite and often small number of functions, the intrinsic mode functions, followed by the application of the Hilbert spectral analysis, to get the signal's instant frequency, phase and amplitude. Hilbert-Huang Transform has been applied to biomedical signals, in particular to the cardiovascular ones. This method provides an accurate estimation of sympathetic and parasympathetic-oscillation powers combined with a dynamic autonomic analysis. The low-frequency band (LF, a marker of sympathetic activity) was computed from the blood pressure variability (LF_{SBP}) while the high-frequency band (HF, a marker of vagal activity) was computed from the heart rate variability (HF_{RRI}).

ii) Baroreflex sensitivity

Baroreflex sensitivity was computed using the sequence method, as described in *Chapter 4*. In brief, this estimation was based on the analysis of beat-to-beat series of sBP, scanned to identify ramps of three or more consecutive heartbeats with a progressive increase ("up-ramp") or decrease ("down-ramp") of at least 1 mmHg, regardless of the possible occurrence of concomitant R-R interval changes. The algorithm identifies spontaneous sequences, defined as sBP ramps, followed by concomitant and concordant RR intervals variations of 5 milliseconds coupled with 0-, 1-, 2- and 3- beat lags, with each sequence being included only once. For each sequence, the slope of the linear interrelationship between sBP and the following RR interval values was calculated and was considered reliable when the correlation coefficient was higher than 0.80 (Parati et al., 1988; Bertinieri et al., 1988; Di Rienzo, Castiglioni, Mancia, Pedotti, & Parati, 2001a; Di Rienzo, Parati, et al., 2001b).

For each period of analysis, the baroreflex effectiveness index (BEI) was defined as the

ratio between the total number of baroreflex sequences detected and the total number of sBP ramp-like changes in blood pressure, regardless of whether the latter is followed by a change in RR-interval or not. The higher the effectiveness value, the more sBP ramp changes are followed by a change in RR-interval.

iii) Cross-Spectral Wavelet Coherence and Phase

Wavelet coherence examines the association of two time series in time and frequency, thus showing whether these two series oscillate simultaneously. High coherence suggests the capability of one time series to predict the following one (Torrence & Compo, 1998; Grinsted et al., 2004; Yang et al., 2008).

In this study, Cross Spectral Wavelet Coherence analysis was performed in a Matlab environment (MathWorks, MA, USA), using our in-lab developed solution, based on the seminal works of Torrence and Compo (Torrence & Compo, 1998) and Grinsted (Grinsted et al., 2004). In brief (Grinsted et al., 2004; Yang et al., 2008), a complex wavelet transform (Morlet wavelet) was used for feature extraction from cardiovascular signals analysed for intervals of 5 minutes. Continuous wavelet transform (CWT) scalograms were calculated for the sBP and the heart period variables. From the two CWTs, a Cross Wavelet Transform (CrWT) was constructed, exposing the common power and relative phase in time-frequency space. Next, the Wavelet Coherence (WTC) between the two CWT was computed. The coherence spectrum, holding values between 0 and 1, is a measure of the correlation between the variations of the two signals for a given frequency. The phase spectrum shows, at each frequency, the phase difference (lead or lag) between the signals, from where the lag period (in seconds) between the two time series was computed.

E. Statistical Analysis

Continuous variables are expressed as mean±standard deviation and plotted as the composite of the mean values of all subjects unless otherwise specified. Categorical variables are given as frequencies and percentage of patients. Normality distribution of the continuous variables was analysed with the Kolmogorov-Smirnov test (Lilliefors'

correction), and Levene's test was used for assessment of homogeneity of variance. Data from the three groups of subjects – fainters, non-fainters and control group - are represented along the time. Student's t-test for paired samples was used to compare all continuous variables' data in the same group, and the chi-square test or Fisher exact test was used in case of proportional differences. Comparison between the control subjects and the adult patients with ('fainters') and without ('non-fainters') HUT-induced vasovagal syncope was made using a "repeated measures" analysis of variance (ANOVA) with one "within" factor (the 5 consecutive periods of analysis) and one "between" factor (fainters vs non-fainters); in the case of significant within-group difference, multiple pair-wise comparisons were made with Bonferroni's correction. All tests were two-sided. Extreme outliers were excluded. The cut-off point for a positive HUT was determined, using receiver-operating curves, as the optimal value that maximises sensitivity and specificity. A value of $p<0.05$ was considered statistically significant. Data were analysed using SPSS software version 23 (IBM Corporation, USA).

III. Results

A. Demographics

Adult Population

Four hundred and seven adult patients were enrolled in this study (age between 18 and 64 years old, mean age 48±12 years old, 59.2% females), having experienced on average 5.2±3.6 syncope episodes before the HUT test. The test was positive in 188 (46.2%) patients, representing the fainters' group. Based on the VASIS classification, 95 patients (50.5%) were defined as mixed type (Type 1) syncope, 52 patients (27.8%) as vasodepressor (Type 3) and finally, 41 (22%) showed a cardioinhibitory (Type 2) profile. Symptoms occurred on average 17.4±5.9 minutes after the beginning of the test. Thirty healthy individuals (mean age 46.8±7.1 years old, 60% females), without a personal history of syncope and with a negative response to HUT test were enrolled as controls. Sex and age distributions of patients and healthy controls were not statistically different. The baseline characteristics of all the adult patients and control group subjects are given in Table 10.

Paediatric Population

Two hundred and thirty-eight paediatric patients were enrolled in this study (age range 12 and 18 years old, mean age 13.4±4.1 years old, 64.3% females), having experienced on average 3.7±2.9 syncope episodes before the HUT test. The test was positive in 99 (41.8%) patients, standing for the paediatric fainters' group. Based on VASIS classification, 38 patients (38%) were defined as cardioinhibitory (type 2), 35 patients (35.4%) mixed type (Type 1) syncope and 26 (26.6%) as vasodepressor (Type 3) profile.

Table 10. Demographics and clinical data of the studied adult population and control group showing patients distribution according to the different types of syncopal episode evoked on HUT.

	All Patients (n=407)	Control group (n=30)	p-value	Patients' groups according to HUT response		P-value
				Fainters (n=188)	Non-fainters (n=219)	
Age (years)	45.7±9.2	46.8±7.1	0.5221	43.4±7.6	46.5±10.4	0.0008
Female gender	241 (59.2%)	18 (60%)	1.0	127 (68%)	118 (54%)	0.006
Body mass index (Kg/m²)	25.8±4.2	25.2±3.4	0.4348	24.5±4.1	26.8±7.2	0.0001
Number of syncope events before HUT	5.2±3.6	0	n/a	7.1±6.4	4.0±2.9	0.0001
≥ 3 syncope events before HUT	218 (53.6%)	0	n/a	122 (65%)	114 (52%)	0.001

B. Haemodynamic response

Mean values for the haemodynamic parameters in both populations, in supine posture and after HUT test, are reported in Figure 21 (and in Table 17, Table 18, and in Figure 36 in Appendix 1). In the supine position, heart rate was significantly higher for both the adults and paediatric fainters group when compared with the non-fainters group and the healthy control one, with paediatric fainters presenting the highest values of HR (78.5±10 vs 85.1±12.3 bpm, respectively; refer to Table 14, Appendix 1). Patients from both groups of fainters also showed significantly lower values of estimated stroke volume and total peripheral resistance and a clear tendency towards lower cardiac output values while in the supine position (Figure 21). No statistically significant haemodynamic differences were found between non-fainters and healthy control subjects (refer to Table 17 in Appendix 1). While none of the remaining haemodynamic data differed significantly amongst the same population, it is noteworthy that, generally, the paediatric population had more extreme values for most of the accountable variables when compared with the adult population (higher HR, and lower values for all the other variables; values from Table 14 in Appendix 1) . Nonetheless, the overall

physiological profile throughout the test was remarkably similar and, based on this, four phases of cardiovascular responses leading to syncope could be described as follows (Figure 22):

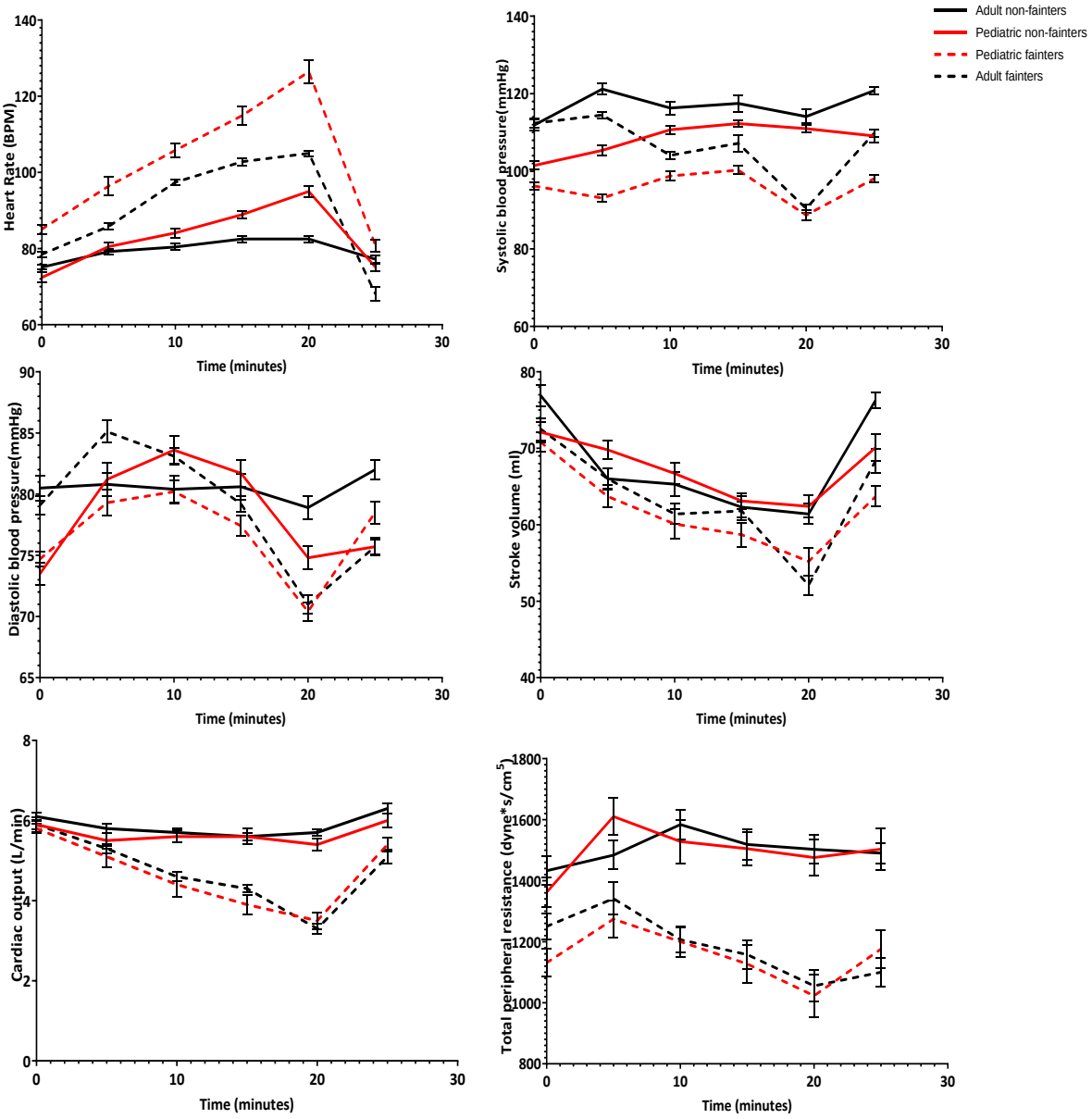


Figure 21. Comparison of the evolution of haemodynamic parameters along time during HUT in the adults and pediatric groups of non-fainters and fainters.

In **Phase 1**, during the first five minutes immediately after assuming the orthostatic position, all subjects were asymptomatic, and sBP was well maintained; an extreme

significant decrease (p<0,0001) of the estimated stroke volume and cardiac output could be seen upon moving from the supine to the orthostatic position. Postural reductions in cardiac output were compensated by a parallel reflex sinus tachycardia and by an increase in peripheral vascular resistance, to maintain blood pressure. While systolic pressure was not significantly different from the baseline, a significant increase in the diastolic blood pressure could be seen (+ 9 mmHg in adults; p< 0.0001). During this phase, heart rate was significantly higher, and sBP, cardiac output and stroke volume were significantly lower in both fainters groups when compared with non-fainters (Figure 21; Figure 36 from Appendix 1). Total peripheral resistance was not significantly different between groups but tended to be lower in the fainters' groups. None of the remaining haemodynamic data differed significantly between groups in phase 1. No statistically significant differences were found between adult non-fainters and healthy control subjects.

In **Phase 2**, on average 6.4±2.7 minutes after the beginning of HUT, and lasting on average around 4.1±2.7 minutes, patients from both fainters groups showed a progressive, but statistically significant, decrease of the estimated cardiac output and total peripheral resistance, with blood pressure being compensated by a significant increase in heart rate (p<0,0001 for both groups), which either continued to increase up to the time when sBP and heart rate became unstable (phase 3) or reached a plateau, which then remained constant for the remainder of the phase. No statistically significant differences were found between the non-fainters and the healthy control subjects (refer to Table 17 in Appendix 1).

Phase 3, from immediately after the end of Phase 2 until the beginning of Phase 4, was characterized by a further decrease of cardiac output compensated by reflex tachycardia (significant increase in HR from 105.8±18.4 to 114.9±25.2 bpm in children, and from 97.4±11.5 to 102.8±13.2 bpm in adults), associated with haemodynamic instability, translated by large oscillations of blood pressure (Figure 21). During this phase, subjects began to experience the first symptoms of pre-syncope. The average amplitude of the oscillations of sBP was 28±3.1 mmHg. The average frequency of the oscillations was 0.1 Hz. Despite not quite significant, the average value of sBP during these oscillations was

slightly higher than the one during the previous phase.

Phase 4 started, on average, three minutes before the syncopal event. Peak heart rate was reached on average 100 seconds before syncope. Indeed, the average heart rate in the adult fainters group was significantly higher than the average values for non-fainters and control groups (105±10.3 vs 82.5±12.6 bpm; $p < 0.0001$; Figure 39 in Appendix I). Similarly, the paediatric fainters group reached a peak of HR (126.4±.30.2 bpm), demarking the most significant gap in HR values, between the two age groups (Figure 21 and Table 14, Appendix 1). This marked tachycardia was followed by a sudden, pronounced, hypotension secondary to further decrease in stroke volume, cardiac output and total peripheral resistance. On average, in all fainters, estimated cardiac output and total peripheral resistance had decreased by 44% and 16%, respectively, when compared with the supine position. Ending the procedure and restoring the subject to a supine position limited the duration and the minimum arterial pressure reached during this phase.

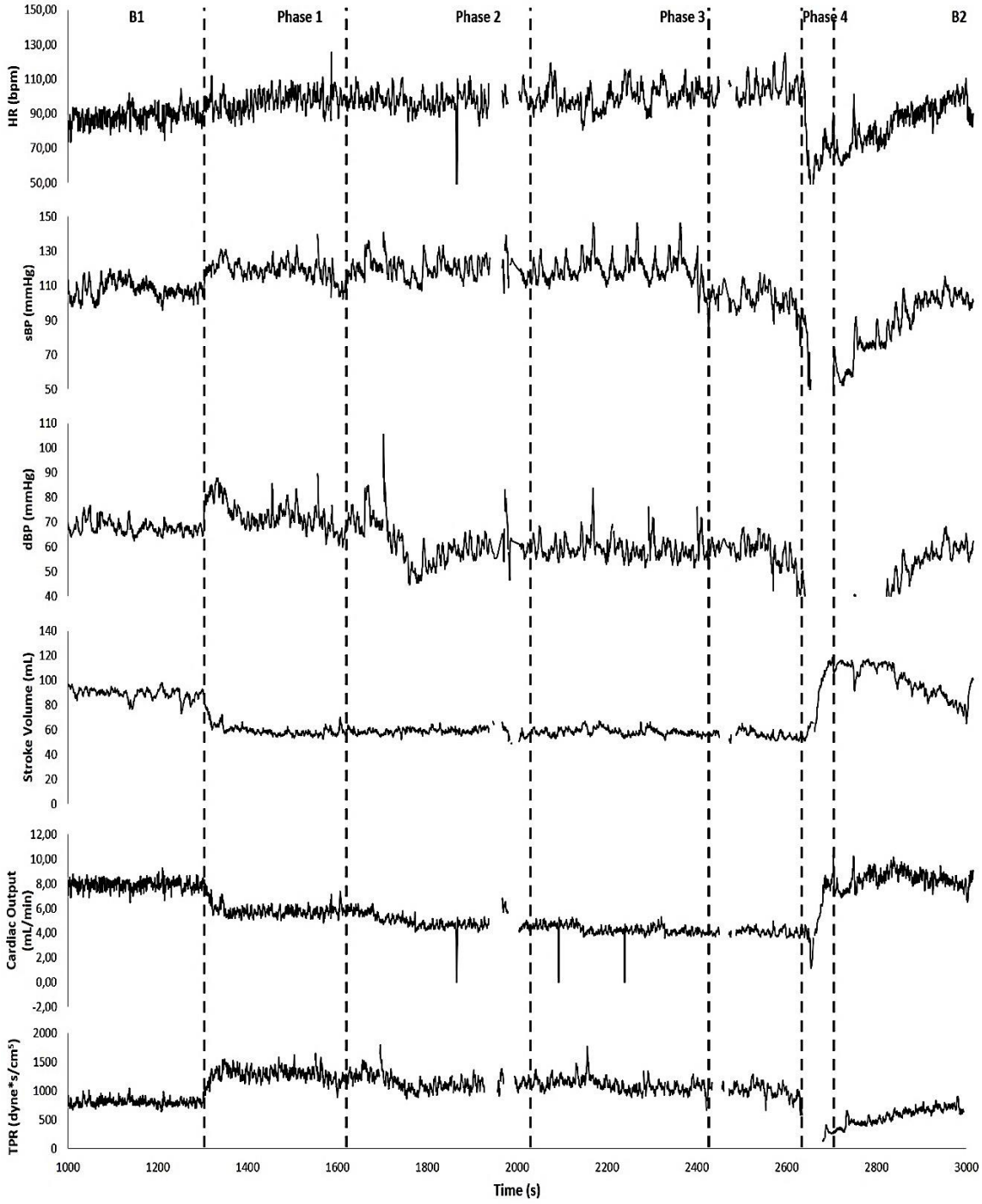


Figure 22. Four Phases of Cardiovascular Response to HUT

Heart rate (HR), systolic blood pressure (sBP), diastolic blood pressure (dBP), stroke volume, cardiac output, and total peripheral resistance (TPRI) of a representative fainting patient during head-up tilt (HUT).

C. Heart Rate and Blood Pressure Variability

Overall, all groups considered herein, i.e. control, adults and paediatric fainters and non-fainters, showed an increase in LF_{SBP} activity during HUT, as demonstrated in Figure 23. However, fainters' response to HUT, when compared with the non-fainters and healthy control subjects, was characterised by a significant rise ($p<0,0001$) in sympathetic activity after tilting (15,4±8,5, 7,8±4,9 and 6,3±5,1 $mmHg^2$, respectively; Figure 37 and Table 17 in Appendix I). The latter was also true for the paediatric population, with even higher values LF_{SBP} (19.4±10.2 $mmHg^2$). In fact, as expected from the profile of the above described haemodynamic response, whilst paediatric fainters presented significantly higher LF_{SBP} values, throughout the HUT test (Table 14 and Table 15 in Appendix 1), the general trace of blood pressure variability, is remarkably similar between the two groups of fainters (Figure 23).

In brief, the core dynamic changes to LF elicited by the HUT included a sudden and initial rise of sympathetic tone immediately after tilting-up (Phase 1), followed by a significant decrease of sympathetic activity (Phase 2), a second overshoot of activity (Phase 3), and, then, a steady fall-off of output 1 to 2 minutes prior to syncope (Phase 4). This pattern, present in every fainter, was absent in all non-fainters and control group subjects (refer to Table 17 in Appendix 1).

As displayed in Figures 23 and Figure 37 (see Appendix 1), both groups of fainters presented significantly lower values of HF_{RRI} band (markers of parasympathetic activity) during the majority of the test, comparing with non-fainters, except after tilting down, where significantly higher values were found in the adult fainters group (591,8±348,1 vs 484,8±348,1, respectively; Table 18 in Appendix 1). In more detail, fainters followed a trend for significant decrease during the first phases of HUT (phases 1 and 2), particularly paediatric fainters, with a steep decrease (from 932,8±620,4 to 259.1±217.4 ms^2, basal and phase 4 respectively). This incredible decrease in parasympathetic activity culminates in a sharp, extremely significant increase during tilt-down, with a p-value under 0,0001. Interestingly enough is the almost 100% overlapping of values during the recovery phase between the two groups of fainters (Figure 23; values in Table 14 Appendix 1).

Finally, the results show a clear tendency for higher LF_{SBP} values and lower HF_{RRI} values on the groups of non-fainters when compared with healthy control subjects (refer to Figure 37 and Table 17 in Appendix 1). No other statistically significant differences were found between non-fainters and healthy control subjects.

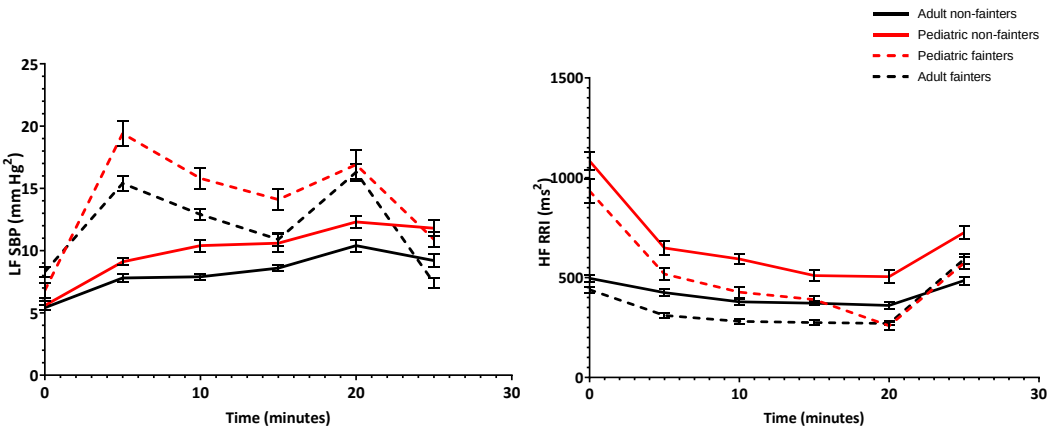


Figure 23. Heart Rate and Blood Pressure Variability. *Comparison of the evolution of heart rate and blood pressure parameters along time during HUT in the adults and paediatric, non-fainters, and fainters groups*.

D. Cardiac baroreflex evaluation

Non-fainters and healthy controls had significantly more sBP ramps than the fainters' group both in the supine position (4.5±2.1 vs 2.8±1.7 SBP ramps /100 heart-beats; p=0.043) and after HUT (6.7±3.1 vs 2.5±1.9 SBP ramps /100 heart-beats; p<0.001). At the same time, while in the supine position, for the non-fainters group, baroreflex sequences represented approximately 20% of the overall analysed sequences (20.6±3.3%), whereas for the fainters they represented only 14±3% of baroreflex sequences (p=0.05). After head-up tilt, there was a small but significant increase in the percentage of baroreflex sequences with HUT in both groups (29±2.4% in non-fainters; 19.7±1.8% for the fainters group).

Sensitivity or Baroreflex Gain

In the supine position, fainters and non-fainters had similar BEI (65±25.7% vs 72±30.2%, p=NS), with a non-significant trend for higher resting BEI values in the fainters group (Figure 24). However, after HUT, the two populations showed different trends. In fact, while the non-fainters group showed an increased BEI till reaching a plateau that was, then, maintained all along the manoeuvre (baseline 65±25.7% vs. plateau 75.7±20.4%, p=NS), the fainters' group showed a progressive but significant BEI decrease (72±30.2% vs 45.9±18.3%; p<0.001) (Table 16).

Delay evaluated by the sequence method

Baroreflex response upon HUT was not constant, leading to modifications on the number of spontaneous sequences, which relates changes of sBP with RR intervals. In the non-fainters and healthy control groups, a lower delay (lag) of the baroreflex response was observed in the supine position, when compared to the fainters' group, with 90% of all spontaneous baroreflex sequences occurring on lag 0. With HUT, the relative predominance of lag 0-, 1- and 2- sequences changed. In fact, despite non–significant, from the total pool of sequences, there was a net reduction of lag 0-sequences, from 90% to 74%, implying an increase of lag 1- and 2- sequences, which corresponds to 26% of all sequences during HUT in non-fainters and healthy controls.

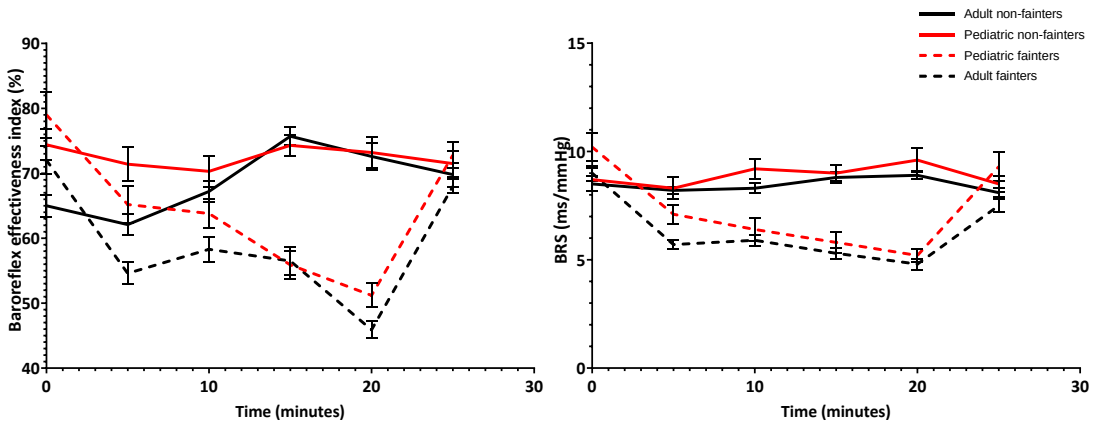


Figure 24. BEI and BRS. *Comparison of the evolution of BEI and BRS parameters along time during HUT.*

On the other hand, the fainters group, showed a significant delay of the baroreflex response, both during supine position and after HUT ($p<0.001$). In this population, while at rest, only 68% of all spontaneous baroreflex sequences occurred with lag 0. With HUT, there was a significant reduction in the number of lag 0 sequences (baseline 68% vs HUT 25%), while lag 1- and lag 2- sequences increased (from 19 to 51% and 13 to 32%, respectively).

E. Wavelet Coherence and Phase analysis

Wavelet Coherence

Coherence between the sBP and RR interval signals occurred both for the LF (0.04 - 015 Hz) and HF (0.15–0.4 Hz) bands, throughout the resting period (Figure 25). In the supine position, non-fainters showed non-significantly higher values of average LF band coherence than the fainters' group (0.75±0.20 vs 0.70±0.17; p=NS). With HUT, a general loss of the spectral coherence between sBP and RR interval spectra occurred for all the assessed individuals. In the non-fainters group, coherence levels reached its minimum value 18±5 seconds after HUT and recovered to a value significantly lower than in supine position (0.60±0.24; p=0.05). For the fainters group, the loss of cross-spectral coherence for the LF band is even more pronounced, reaching 0.35±0.12 ($p<0.001$) immediately before the syncopal event. This decrease in coherence power meets the results obtained with the baroreflex sequence method.

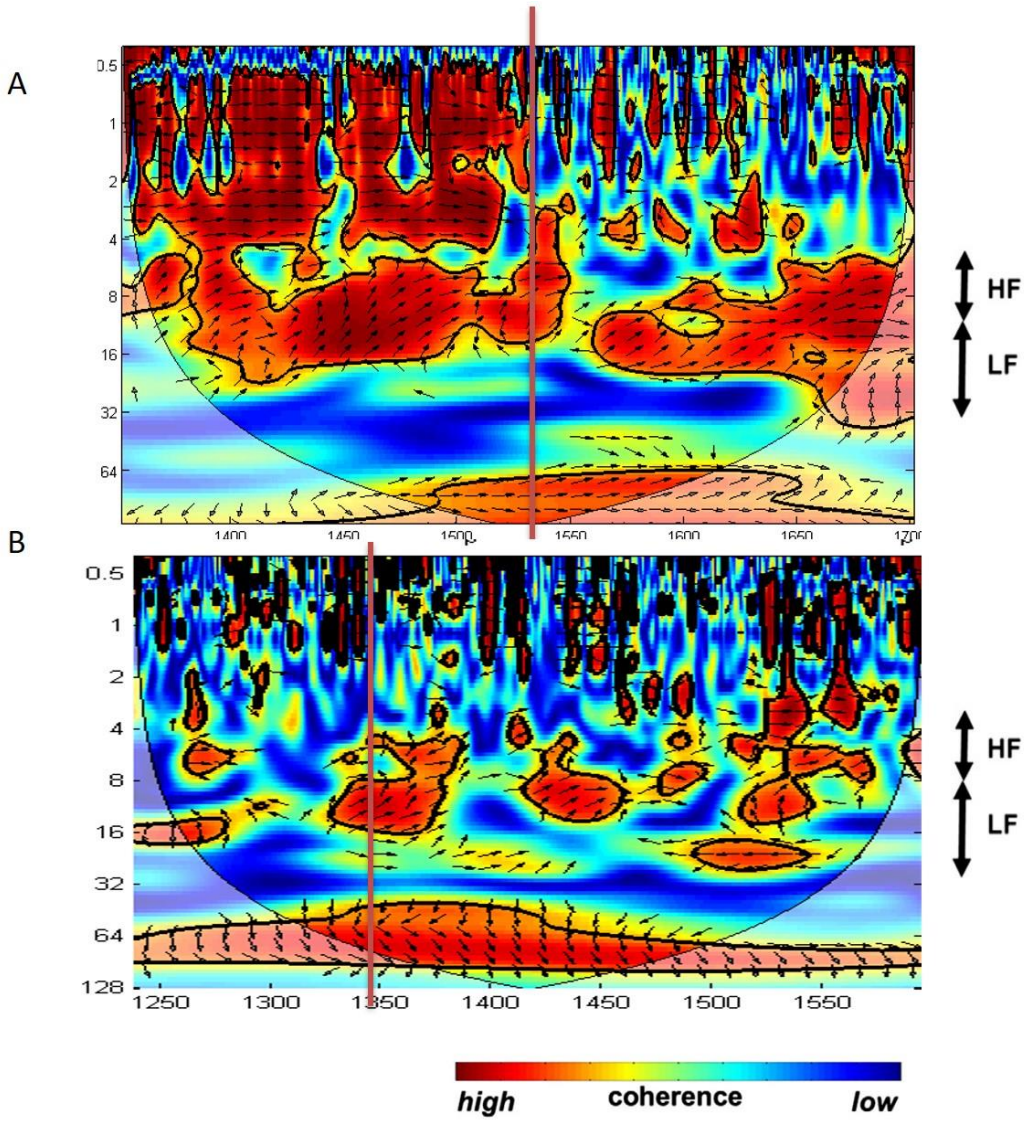


Figure 25. Wavelet coherence plot of a control individual (A) and a fainter (B).
The red vertical line marks the start of the head-up tilt. Arrows represent the instantaneous phase in patients with reflex syncope. In the control individual, after tilting (vertical line), there is a drop of coherence, which reaches its minimum value approximately 20 seconds after tilting, recovering later. In this example, concerning the fainter, a significantly lower coherence could be documented during the duration of the manoeuvre.

Phase analysis and time delay

The time delay in seconds, derived from the phase analysis, showed that for the non-fainters and healthy controls group, sBP and RR-intervals LF spectral bands oscillate with mean synchronicity of 0.75±0.30 seconds which, after HUT, increased to an average delay of 1.0±0.24 seconds, with a maximum delay below 1.5 seconds. On the other hand,

the fainters group showed a significantly increased delay between both spectral bands, either during supine position (from ~1.0 seconds to 1.5 seconds, p=0.05) or after HUT (> 1.5 seconds, p<0.001).

d) Non-fainters vs healthy controls groups' responses

Interestingly, non-fainters and healthy controls did not show statistically significant differences for all the evaluated parameters, despite the different clinical presentation of both groups of individuals. These observations allow speculating about the non-reflex/non-orthostatic origin of the syncopal events observed in the non-fainter patients.

F. Predicting Head-up Tilt table outcomes

Multivariate analysis revealed a significant overall difference in the HUT table test outcome as a function of delay of baroreceptor response, baroreflex effectiveness index and the power of the LF_{SBP} band assessed in the first five minutes of HUT. All variables had a significant impact on the tilt outcome and on the time of symptom onset, with those with lower baroreflex effectiveness index (BEI <58.5%; sensibility 85.53; specificity 96.8; AUC 0.9157; p<0.0001), higher delay of the baroreceptor response (> 1.45 s; sensibility 88.3; specificity 88.13; AUC 0.8596; p<0.0001) and a higher LF_{SBP} power (>12.25mmHg^2 ; sensibility 84.57; specificity 90.69; AUC 0.9485; p<0.0001) being more likely to have positive HUT table response (Figure 26).

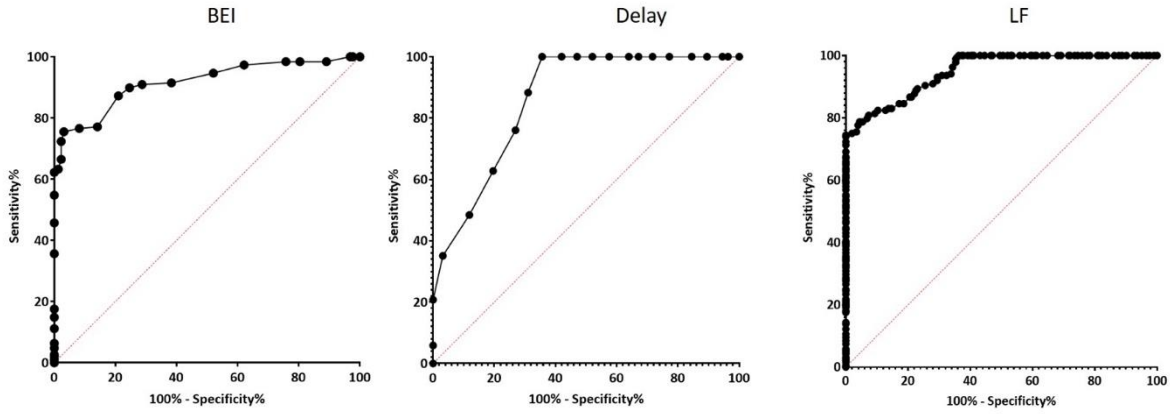


Figure 26. ROC curves for the BEI, delay of the baroreceptor response and LF_{SBP}

IV. Discussion

The significance of the baroreceptor response in initiating compensatory reflex responses has been thoroughly described (Thomson et al., 1997; Iacoviello et al., 2010; Laranjo et al., 2015; Jardine et al., 2018). On the other hand, vasovagal syncope is a prime example of autonomic dysregulation, with the development of profound hypotension and/or bradycardia, traditionally associated to a withdrawal of vasomotor sympathetic neural traffic and/or neurohormonal vasodilation. Despite all the available literature, the pathophysiology of vasovagal syncope is still just partially understood.

The major findings and conceptual contribution of our work lead to the acknowledgement that the dysregulation of the hemodynamic homeostasis observed through the autonomic and baroreceptor reflex behaviour upon an orthostatic stress test is a reliable clinical tool to stratify reflex syncope patients, in order to define an integrated and personalised therapeutic scheme. Our results showed that recurrent reflex -syncope patients having a positive response to HUT (fainters) revealed a significantly prolonged delay in the baroreceptor mediated RR- interval response to the orthostatic position when compared to healthy individuals and non-fainters. Results from this study will be directed to build predictive tools to be used by clinicians and patients as complementary measures to improve symptoms.

A. Haemodynamic behaviour

Overall, our results, both in adult and paediatric patients, indicate that before HUT-induced syncope there is a steep fall in cardiac output, immediately after HUT (*Phase 1*), associated with a transient increase of total peripheral resistance. A progressive, non-significant, decrease of total peripheral resistance was seen starting in *Phase 2* and extending until *Phase 4*, when a second significant fall in cardiac output, associated with a significant decrease of total peripheral resistance, was observed. In agreement with these results, we documented a 44% reduction in cardiac output, from 5.9±1.5 L/min, in the supine position, to 3.3±1.8 L/min at the time of syncope, associated with a smaller percentual decrease of total peripheral resistance (16%).

There has been much debate as to whether a decrease of the cardiac output or the total peripheral resistance is the dominant hypotensive mechanism preceding vasovagal syncope (Wieling et al., 2016). Nevertheless, the reasons for a decreased cardiac output preceding HUT-induced vasovagal syncope are uncertain and probably multifactorial. Impairments in venous return, peripheral vasodilation and blood pooling, autonomic function and neuroendocrine cardiovascular control and finally, dysfunctional arterial baroreflex function, may all play a role in this behaviour.

Impairment of vasoconstrictive responses has been reported in syncope patients (Sneddon, Counihan, et al., 1993b; Thomson et al., 1997). However, exaggerated vascular responses have also been demonstrated during the application of sub-hypotensive lower body negative pressure (Sneddon, Counihan, et al., 1993a; Flevari et al., 2002). Thus, an alternative hypothesis considered was that the cardiac output fall is the predominant physiological event that determines the observed hypotension. Such a fall in cardiac output prior to syncope has previously been demonstrated using the thermodilution method (Jardine et al., 2002) and, was later confirmed by the non-invasive pulse pressure-based method (Verheyden, Liu, et al., 2008; Fu et al., 2012). These studies demonstrated that vasovagal syncope is likely to occur when cardiac output is approximately decreased to half of its initial value (Verheyden, Liu, et al.,

2008). In our study, changes in both parameters were observed but at different time points.

B. Autonomic nervous system behaviour

In the present work, we used non-stationary methods for heart rate variability analysis, in-house-developed (to details please refer to *Chapter 4*) and validated in other typology of patients and in healthy subjects (Ducla-Soares et al., 2007; Tavares, Martins, Oliveira, Laranjo, & Rocha, 2012), to study the heart and blood pressure variability profiles during drug-free HUT in patients with recurrent episodes of vasovagal syncope and in a group of healthy individuals. All subjects showed an increase in LF- a spectral marker of sympathetic activity - during HUT. Moreover, fainters' response to HUT (both in adult and paediatric patients), when compared with the non-fainters and healthy controls, was characterised by a significant rise in sympathetic activity after tilting. For these patients, LF changed dynamically during HUT, showing a sudden and initial rise of sympathetic tone immediately after tilting-up (*Phase 1*), followed by a significant decrease of sympathetic tone (*Phase 2*), a second overshoot of activity (*Phase 3*), continued by a steady fall-off of output 1 to 2 minutes prior to syncope (*Phase 4*). This pattern occurred in every fainter, regardless of the type of syncope response and age group.

The maximum increase in sympathetic nerve activity from baseline to orthostatic intolerance reflects the physiological reserve for sympathetic activation of the heart. The sudden initial rise of sympathetic tone immediately after HUT in the fainters group may exhaust their reserve to increase sympathetic modulation of vasomotor tone and peripheral resistance any further during the remaining of the test (Sneddon, Counihan, et al., 1993b; Lambert & Lambert, 2014), leading to a non-effective arterial baroreceptor reflex action, and, thus, culminating in syncope. On the other hand, high-frequency values (HF, markers of parasympathetic activity) showed a trend for a significant decrease during the first 10 minutes of HUT (*phases 1* and *2*); fainters presented significantly lower values of HF band during the majority of the test, except after tilting

down, where significantly higher values were found in the fainters' group. These results are in line with those of other authors (Jardine et al., 1998) who showed that at the onset of HUT arterial baroreceptor sensitivity was reduced, but blood pressure was maintained by an appropriate increase in sympathetic activity. "The mechanism for the onset of presyncopal hypotension remains unknown, but it is not due to a reduction in muscle sympathetic nerve activity (MSNA) nor an increase in cardiac parasympathetic activity. Presyncope and syncope appeared to be mediated by a sympathetic withdrawal, resulting in vasodilation and bradycardia with no evidence of a reciprocal increase in parasympathetic activity" (Jardine et al., 1998). In addition, it was also shown that, at the onset of syncope, LF (nu) suddenly dropped to nearly zero, thus suggesting a persistent predominance of the sympathetic drive to the heart, up to the vasovagal event (Furlan et al., 1998). In those subjects, the neural control of the sinoatrial node activity followed a peculiar time course, characterised by an increase of cardiac sympathetic modulation in the early phase of HUT, compared with the supine position and, in most cases, followed latter-on by its further enhancement in the period immediately preceding fainting onset. The vagal modulation of heart period, inferred from the HF (nu) power, decreased during the last minute that preceded syncope. In an elegant study, Kamiya and colleagues determined the temporal occurrence of events preceding presyncope. During central hypovolemia induced by HUT, both the absolute levels of MSNA (expressed as burst frequency and total activity) and the amplitude of LF oscillations increased markedly; further, as subjects moved toward presyncope, LF oscillations, derived from both blood pressure and MSNA, decreased and were associated with a drop in mean arterial blood pressure, despite the maintenance of MSNA burst frequency and total activity at their elevated level (Kamiya et al., 2005; Ryan, Rickards, Hinojosa-Laborde, Cooke, & Convertino, 2012).

Over the last twenty years, several studies were published focusing on HRV analysis in the evaluation of autonomic activity in patients with vasovagal syncope. Such studies tried to meet two primary aims: to build a better knowledge about the autonomic mechanisms leading to vasovagal syncope and to provide new diagnostic tools able to identify patients prone to such reactions. The available literature is often controversial, and sometimes conflicting regarding the role of both efferent limbs of autonomic

nervous system reflex arc in the genesis of vasovagal syncope (Jardine et al., 1998; Kochiadakis et al., 1998; Furlan et al., 1998; Moak, Bailey, & Makhlouf, 2002).

Based on experimental observations in non-fainting healthy volunteers, it was at first proposed that an initial exaggerated sympathetic activation, in combination with relative ventricular hypovolemia, would result in reflex vasodilation with or without bradycardia (Mark, 1983; Abboud, 1989; Waxman, Cameron, & Wald, 1993; Mosqueda-Garcia et al., 1997). Presently, this theory has globally been dismissed due to experimental evidence. In particular, and using echocardiographic measurements of left cardiac chamber size and determinations of stroke volume, Novak and co-workers did not find any evidence of progressive excessive cardiac emptying before syncope onset in susceptible patients (Novak et al., 1996). Furthermore, Fitzpatrick and collaborators reported the induction of vasovagal syncope by HUT in two patients with orthotopic heart transplantation and absence of nervous connections between the heart and the brain, (Fitzpatrick et al., 1993; Blanc & Benditt, 2016). Some other studies suggested that the vasodilation observed in vasovagal syncope results from a withdrawal of sympathetic tone (Weiss, Wilkins, & Haynes, 1937; Barcroft, Edholm, Mcmichael, & Sharpey-Schafer, 1944). This explanation was supported by microneurography studies in healthy subjects undergoing infusion of vasodilator agents (Wallin & Sundlöf, 1982; van Lieshout et al., 1991) or during pronounced lower body negative pressure (Sanders & Ferguson, 1989), where a common initial observation was the almost complete withdrawal of MSNA during syncope, inferred as a total withdrawal of vascular sympathetic tone. This view has recently been challenged (Vaddadi et al., 2010; Blanc & Benditt, 2016) due to the persistence of MSNA during syncope in most patients who developed vasovagal syncope during HUT test, suggesting that a loss of sympathetic nerve activity may not drive the cascade of syncopal events. Fu and co-workers made further reinforcement of this rationale by showing that sympathetic withdrawal occurs late, after the onset of hypotension in healthy individuals who developed presyncope during head-up tilt (Fu et al., 2012).

The reasons for these inconsistencies on the autonomic involvement in reflex syncope are multifactorial. First, the populations studied are heterogeneous in terms of age, the

number of recurrences of syncope and the used diagnostic test protocol. Furthermore, not all of them adopted the same methodology of autonomic nervous system assessment, namely HRV analysis method, in terms of signal duration, pre-processing or signal analysis algorithm. Regarding the evaluation methodology, a significant limitation of the classical spectral analysis is the steady-state assumption. Analysis of HRV during head-up tilt is complicated, owing to instability periods and data interpretation problems, thus, leading to the contradictory results and conclusions of the studies mentioned above. Non-stationary processing is essential because, in the cardiovascular system, stationarity is a rare exception rather than a rule (Orini, Laguna, Mainardi, & Bailón, 2012). This might be one of the most crucial factors influencing the results and their interpretation.

As controversial as the autonomic changes leading to syncope is their (patho) physiological significance. It has been suggested that the sudden decrease in sympathetic tone and the relative predominance of parasympathetic activity, may protect the heart from the well-known adverse effects of excessive sympathetic drive in the setting of exposure to a stressful condition, such as syncope and its manifestations (Alboni, Alboni, & Bertorelle, 2008; Blanc & Benditt, 2016). This is in line with the integrated behaviour of both systems to maintain homeostasis or allostasis: the sympathetic system acting as a phasic system addressing episodic events, whereas the parasympathetic system acts with a tonic behaviour, maintaining the essential physiological functions.

C. Baroreceptor Reflex Behaviour

The analysis of baroreflex sensitivity (BRS) enables to qualify and quantify the circulatory effects of reflex nature due to baroreceptor excitation or inhibition and is an indirect way of evaluating the changes in autonomic tone. Within this study, we documented a reduction of global baroreceptor responsiveness accompanying an evolving vasovagal syncope. Thus, vasovagal syncope seems to be associated with a transient functional change in the arterial baroreceptor reflex, and this change may-partially account for the

absence of an adequate compensatory response in the setting of specific haemodynamic circumstances.

Despite considerable efforts and extensive research along the last three decades, the available evidence for the role of baroreflex abnormalities in patients with vasovagal syncope is inconsistent. Findings consistent with changes in baroreflex function predisposing to vasovagal syncope have been suggested by different studies, most of which have not found any compelling evidence of alterations in the arterial baroreflex control of heart rate in tilt-induced syncope patients, although a few reported either a reduction or an increase in baroreflex activity (Lagi, Cipriani, Fattorini, Paggetti, & Macerata, 1994; El-Sayed & Hainsworth, 1995; Morillo et al., 1997; Mosqueda-Garcia et al., 1997; Ellenbogen et al., 2006)(Sneddon, Bashir, et al., 1993; Morillo et al., 1997; Thomson et al., 1997; Lipsitz, Morin, Gagnon, Kiely, & Medina, 1997; Jardine et al., 1998; Freitas et al., 1999). There are several reasons for these conflicting findings, namely a) the methods used to induce orthostatic stress (e.g., HUT table test vs lower body negative pressure chamber); b) the differences in HUT table protocols; c) the different methods of measuring baroreflex sensitivity, such as the Oxford technique (Hunt, Fahy, Farquhar, & Taylor, 2001), the sequence method, or direct measurements of sympathetic nerve activity using microneurography; d) the assessment of arterial baroreflex function at different phases of HUT, i.e., at baseline, during or after HUT, immediately before syncope; e) the criteria of patient enrolment; and, f) the heterogeneity of pathophysiological pathways. In addition, the interpretation of these results should also account for the fact that heart rate changes are only a part of the complexity of the reflex circulatory response to short-term blood pressure fluctuations, in which the systemic vascular resistance control seems to be the primary target (Sosnowski, 2010).

Various methods have been proposed to evaluate BRS, all of them based on the relationship between the changes of the heart period and changes of the sBP. Besides stimulus-dependent methods (phenylephrine infusion, Valsalva manoeuvre, neck chamber technique), an analysis of spontaneous BRS is also available (Robbe et al., 1987; Pagani et al., 1988; Barbieri, Parati, & Saul, 2001; Laude et al., 2004; Gouveia, Rocha,

Laguna, & Lago, 2009; Vallais, Baselli, Lucini, Pagani, & Porta, 2009). Each approach has its pros and cons. In general, invasive methodologies allow for the assessment of baroreflex function under standardised, controlled conditions and supply information of proven physiological and clinical value. However, the non-physiological nature of laboratory stimuli (such as vasoactive agents' injection or methods based on mechanical manipulations, e.g. lower body negative pressure chamber) cannot yield useful information on daily life behavioural modulation of BRS.

Two principal non-invasive methods have been proposed, so far, for the analysis of spontaneous BRS: the spectral method and the sequence method (La Rovere, Pinna, & Raczak, 2008). The sequence method, described in detail both in *Chapter 4* and in the present chapter, is based on the spontaneous presence of concurrent R-R interval and sBP changes (increase and decrease) over at least three consecutive heartbeats (sequence) [(Parati et al., 1988; Di Rienzo, Castiglioni, et al., 2001a; Sosnowski, 2010)]. Lower BRS values have commonly been observed in comparison with the phenylephrine test (Parati et al., 1990; La Rovere et al., 2008). In our study, we used the sequence method in its last adaptation (Di Rienzo, Castiglioni, et al., 2001a; Di Rienzo, Parati, et al., 2001b). The sequence method allows for the assessing of two parameters that provide complementary information concerning the baroreflex control of heart rate: BRS offers qualitative information about baroreceptor sensitivity by measuring the magnitude of the reflex changes in R-R interval in relation to the amplitude of the reflex changes in the sBP (Iacoviello et al., 2008); the baroreflex effectiveness index (BEI) provides quantitative information about arterial baroreflex function by quantifying the number of times the baroreflex is effective in driving the sinus node (Di Rienzo, Parati, et al., 2001b; Iacoviello et al., 2008, 2010). Advantages of the spontaneous BRS evaluation include better reproducibility in comparison to stimulus-dependent methods and the fact that it can be used to evaluate short time periods, allowing the assessment of the dynamics of the baroreceptor reflex activity. In this way, it is possible to measure BRS during different phases of the HUT test. It is a less time-consuming methodology, which is not operator-dependent (La Rovere et al., 2008).

The assessment of the arterial baroreflex in terms of its sensitivity has received considerable clinical attention. However, the stability of a physiological control system, such as the arterial baroreflex, depends not only on the magnitude of the effector response (i.e., sensitivity or gain), but also critically relies on the timing of this response (i.e., delay or latency) (Mackey & Glass, 1977; Cavalcanti & Belardinelli, 1996a; Cavalcanti, Severi, & Boarini, 1996b; Gulli, Cooper, et al., 2005). In the last few years, several new algorithms for BRS have been developed. These methods and their improvements have the capability of following changes in non-stationarity conditions and allow for the estimation of the prevailing direction of the coupling between heart rate and blood pressure variabilities. The assessment of the prevailing direction of the coupling is relevant because it can be used to infer which mechanism is primarily responsible for the changes observed in the signals and is necessary to assess spontaneous BRS (Porta et al., 2011). In the present work, to estimate the physiological coupling and to determine the prevailing direction between heart rate and blood pressure, we used a Wavelet Cross-spectral Coherence and Phase analysis algorithm. Our primary purpose was to characterise the dynamic interactions between systolic arterial blood pressure and R-R intervals, including both the assessment of the strength and prevailing direction of local coupling, phase differences and the time-delay between the two signals.

Wavelet coherence was first used for dynamic analysis of the respiratory sinus arrhythmia (Keissar, Davrath, & Akselrod, 2006). Coherence is a complex function of the frequency and estimates a linear correlation between two signals (Sosnowski, 2010). Availability of the cross-spectrum analysis allows for the calculation of the phase relationship in the range of -180^{o} and $+180^{o}$, with a positive angle for a leading x(t), and a negative angle for a leading y(t), allowing for the calculation of the phase coordination when there is a temporal or a phase incidence between blood pressure and heart rate variabilities and phase synchronization which indicates the physiological coupling between signals (Bettermann, Amponsah, Cysarz, & van Leeuwen, 1999; Galletly & Larsen, 2001).

Next, we will discuss the arterial baroreflex patterns during HUT separately, first in the healthy control and non-fainter subjects and then in the fainters.

a) Healthy controls and non-fainters' profiles

In our series, for these two groups of subjects, a lower BRS and BEI value was documented on the supine position, followed by an initial and slight decrease of their values after orthostatism, with a progressive improvement in both BRS and BEI throughout the HUT. Our finding that BRS decreased with HUT compared to the supine position is in line with previous work (Steptoe & Vögele, 1990; O'Leary, Kimmerly, Cechetto, & Shoemaker, 2003; Nollo, Faes, Porta, Antolini, & Ravelli, 2005; Faes, Nollo, & Porta, 2013; Schwartz, Medow, Messer, & Stewart, 2013; Zamunér et al., 2015; Silvani et al., 2017). It has also been shown that cardiac baroreflex sensitivity, at the baroreflex operating point, decreases during HUT, and is accurately tracked by estimates of spontaneous BRS (Schwartz et al., 2013).

Regarding baroreflex latency, the majority of baroreflex sequences while in the supine position occurred within lags 0 and 1. These results are in line with the published literature. It has been shown that, when the relationship between sBP and R-R intervals in response to a rapid blood pressure change is linear, the quantitative measure of BRS is provided by the slope of the regression line fitting changes in sBP against the second succeeding R-R interval (one-beat delay)[(deBoer, Karemaker, & Strackee, 1987; La Rovere, 1999)]. Upon HUT, there was an increase in the relative frequency of sequences occurring at lags 1- and 2-, which translates an increase in the average delay of the chronotropic response. The same results were made further clear with the cross-spectral wavelet coherence analysis, where a transient decrease in the coherence values and an increase in the latency of the baroreflex, from an average of 0.75 seconds while in supine position to 1 second upon tilt, was seen during the first 5 minutes of orthostatism, followed by an increase of the coherence and normalization of the delay up until the end of the manoeuvre. The tilt-induced increase of the lag of the baroreflex chronotropic response to a change in the systolic arterial blood pressure input, suggests an activation of the slow sympathetic regulation simultaneously with the deactivation of the fast-vagal response (Malliani, 2000; Nollo et al., 2005).

The average latency of 0.75 seconds at rest is compatible with the theoretical time of baroreflex latency previously described in the literature, validating this analytical methodology. Previous human and animal studies on baroreceptor regulation of blood pressure have reported a baroreflex latency around 500 - 800 ms (Koepchen, Lux, & Wagner, 1961; Bevegård, Jonsson, & Karlöf, 1967; Pickering & Davies, 1973; Fisher et al., 2009). The measurement of the time relation between the rise of arterial pressure provoked by phenylephrine and the resulting reflex bradycardia showed various conclusions (Pickering & Davies, 1973). Indeed, for heart rates under 75 bpm, the best correlation was found when each beat was related to the immediately following pulse interval (lag 0). For faster heart rates (>75 bpm), the correlation was better if each pressure was related to the next-but-one pulse interval (lag 1). These results allowed the estimation of the conduction time for the whole reflex loop of ~775 ms. These results are also in accordance with other authors (Koepchen et al., 1961; Bevegård et al., 1967). Using a neck chamber, Fisher and co-workers studied the carotid baroreflex peak response latency in humans (Fisher et al., 2009) and found an age-related delay in the cardiovascular response to hypertension, but a similar temporal response to hypotension simulated with neck pressure. Furthermore, the heart rate responses to changes in arterial blood pressure were quite rapid, occurring in 1 second.

In our study, using a wavelet coherence and phase synchronisation algorithm, the time delay of the cardiac baroreflex response in seconds for the non-fainters and healthy control groups was of 0.75±0.30 seconds in the supine position which, after HUT, increased to an average delay of 1.0±0.24 seconds, with a maximum delay below 1.5 seconds. These results are in line with those observed in earlier studies (Cooke et al., 1999; Porta, Baselli, Rimoldi, Malliani, & Pagani, 2000; Nollo et al., 2005). For example, Orini and colleagues assessed cross-spectral coherence and phase synchronization in healthy individuals subjected to HUT test (Orini, Laguna, et al., 2012); in their study, for the LF range, the phase differences were about −0.60 ± 0.11 rad, revealing that a change in the LF oscillation of sBP variability preceded a correlated change in the LF oscillation of heart rate variability by ~875±190 ms. Upon the HUT test, the level of coherence transiently decreased, recovering in around 2 minutes.

b) Patients with head-up tilt induced syncope ('fainters')

In the present study, we used stringent and well-defined inclusion/exclusion criteria, which resulted in the selection of a remarkably homogeneous population of otherwise healthy subjects, with a history of recurrent syncope, without any confounding interference which might have influenced HUT tilt test outcomes or the autonomic control of heart rate. The main finding of our study is that patients with recurrent unexplained syncope who are prone to experience vasovagal syncope during HUT table test ('fainters') demonstrate an abnormal baroreceptor reflex function during HUT. This abnormal function was characterised by both a qualitative (BRS) and quantitative (BEI) impairment, associated with an increased latency for the baroreceptor induced chronotropic response, assessed both by the sequence method and by cross-spectral wavelet coherence.

In the present work, 'fainters' exhibited a markedly reduced BRS and BEI, starting on a very early phase HUT, when compared with the supine basal levels, while non-fainters and healthy individuals showed a slight, non-significant, decrease in BRS and BEI followed by and progressive improvement of both parameters. Our results, despite the application of the different methodology of evaluation, are in accordance with previous studies that observed the same profile of physiological changes (Mosqueda-Garcia et al., 1997; Flevari et al., 2002; Franchi et al., 2003).

The differences found in BRS and BEI in the patients prone to tilt-induced syncope seem to be related to group differences at rest. As previously demonstrated (Pitzalis et al., 2003; Iacoviello et al., 2008), in our series of patients baseline BRS and BEI values were higher in the fainters than in non-fainters and healthy control individuals. These results are also similar to others using the sequence method (Chaddha et al., 2016). A similar relationship was also noted between BRS and type of HUT response, with higher BRS values noted in patients with tilt-induced asystole and lower BRS values found in patients with mixed and vasodepressor responses. The physiological significance of these observations is still unknown.

Using the same methodology, other authors failed to demonstrate significant differences in the BRS and BEI between tilt positive and tilt negative individuals during the drug-free phase of the manoeuvre (Iacoviello et al., 2008; Mitro, Simurda, Evin, Murin, & Muller, 2015). At the time of syncope, however, BRS was lower in tilt-positive patients compared with tilt-negative patients. Contrary to these authors, we found significantly lower BRS and BEI values in the fainters during the drug-free HUT test and, similarly to a subsequent study from Iacoviello (Iacoviello et al., 2010), we also found that reduced BRS and BEI during HUT was an independent predictor of tilt response, as well as the time to symptom onset. These findings support the hypothesis that impaired BRS may play a vital role in the pathogenesis of vasovagal syncope. The simultaneous impairment of BRS and BEI suggest that in patients with recurrent reflex syncope, HUT induces a qualitative and quantitative worsening in arterial baroreflex function. This could favour the occurrence of vasovagal syncope by leading to an unstable relationship between sBP and R-R interval (Julu et al., 2003) due to the failure of effective arterial baroreflex control.

While it is believed that altered BRS may predispose toward abnormal sympathetic and parasympathetic responses to orthostasis, the mechanism through which baroreceptor control becomes inefficient and uncoupled is not fully understood (Flevari et al., 2002). This might be due to factors that regulate aortic baroreceptor reflex. In fact, previous studies, upon the manipulation of the efferent sympathetic limb of the baroreflex in some of these syncope patients, confirmed that an enhancement in overall sympathetic tone would reduce orthostatic intolerance and, ultimately, prevent syncope induced by tilt (Mosqueda-Garcia, Furlan, Fernandez-Violante, Snell, & Robertson., 1996). These findings, taken together, support the concept that impairment of baroreflex function is a mechanism that leads to abnormal sympathetic responses to standing in susceptible individuals.

Herein, we hypothesised that the mechanism behind this inefficiency could be due to an autonomic impairment characterised by a prolonged time delay to the baroreflex response. Our results have shown that the subjects more able to tolerate orthostatic

stress are the ones who show shorter latency, which would be a characteristic precise response of the ANS in healthy individuals. In the fainters, the majority of baroreflex sequences were coupled with more than two beats of delay, particularly during HUT, where the majority of the baroreflex induced chronotropic responses took more than 2 seconds to occur. Considering the mechanisms behind the prolonged latency in the baroreflex response, the most likely explanation for this behaviour is the dynamicity of the autonomic changes occurring upon tilt. It has been suggested that the mechanism for this lengthy response is the increase in cardiac sympathetic and/or reduction in cardiac parasympathetic tones that are elicited by these stimuli. In previous works, reflex activation of the sympathetic nervous system has been postulated to slow the cardiac baroreflex latency (Sundblad & Linnarsson, 1996). Thus, the increment of the sympathetic nervous system activity (as assessed by the LF band variability), both in the basal supine position, as well as the marked increase after HUT noticed in the fainters, may potentially contribute to prolonged latency of the baroreflex function.

In our study, adult fainters showed a significantly increased delay, either during supine position (from ~1.0 second to 1.5 seconds, $p=0.05$) or after HUT (> 1.5 seconds, $p<0.001$). Our results are in accordance with the ones from Gulli et al. (Gulli, Claydon, et al., 2005). These authors showed, in patients with poor orthostatic tolerance, a prolonged latency between the spontaneous fluctuations of blood pressure and R-R interval, as well as a slower central oscillating frequency of the LF variability, which may also translate an increased delay of the baroreflex response (Gulli, Wight, Hainsworth, & Cevese, 2001; Gulli, Cooper, Claydon, & Hainsworth, 2003). In a later study, the same authors demonstrated a prolonged latency in the baroreflex-mediated vascular resistance response in subjects with posturally related syncope (Gulli, Cooper, et al., 2005). In these patients, the maximal response after baroreceptor unloading was around 3-4 seconds slower than in the controls and patients with normal orthostatic tolerance.

D. Age-related differences

Haemodynamic changes associated with syncope have been found to differ according to age. In our series, older patients are more likely to have a mixed or vasodepressor response compared with younger patients, who are more prone to cardioinhibitory and mixed forms of syncope. The younger fainters were also more prone to an initial exaggerated tachycardic response to HUT, which may represent a marker of cardiovascular instability and account for the occurrence of large oscillations in heart rate and sBP observed before the onset of syncope (Lipsitz et al., 1997; Pitzalis et al., 2002). Besides, younger patients had higher values of heart rate and blood pressure variability, both in the basal supine position as well as during HUT, and showed increased absolute values of BRS and BEI, a finding in accordance with previous work (Gribbin, Pickering, Sleight, & Peto, 1971). Despite these quantitative differences, no qualitative difference was found in the dynamic trends of heart and blood pressure variability and baroreflex function during tilt when comparing the younger with, the older patients.

E. Mechanism of head-up tilt-induced syncope

Assessing and integrating all data from this study, we consider that a primary dysfunction of the ANS underlies reflex syncope, having as a core manifestation an impairment of the arterial baroreceptor reflex, which is not able to compensate for the fall in blood pressure.

The decreased BRS and BEI, coupled with a prolonged latency of the baroreflex, might explain the inability of these subjects to increase promptly sympathetic outflow in response to reductions in pressure. Indeed, due to this increased latency, when blood pressure falls after assuming the orthostatic position, the physiological compensatory responses, even when of appropriate magnitude, may come too late, after the cerebral perfusion pressure has passed beyond the critical “pre-syncope/ syncope” level.

The arterial baroreceptor reflex is a negative feedback system aiming at keeping the arterial blood pressure stable during changing physiological conditions. It can be thought of as a closed-loop negative feedback system, with multiple blocks standing for

the major physiological variables. Under most conditions, a normally functioning baroreceptor system would be expected to compensate for decreasing systemic pressure by increasing the heart rate and initiating increased vasoconstriction. In vasovagal syncope, however, the baroreceptor feedback mechanism either fails to counteract evolving hypotension or is only partially effective, resulting in blood pressure and heart rate oscillations.

Computer simulations of baroreflex regulation have demonstrated that both an increase in the latency, as well as a decrease of the gain, of the effector response may have a significant impact on the stability of the system by generating an unstable state of regulation, where cardiovascular oscillations become more complex and chaotic (Madwed, Albrecht, Mark, & Cohen, 1989; Keyl, Schneider, Dambacher, & Bernardi, 2001). Thus, it is possible that an increased time delay of the baroreflex response, as we observed in fainters, may contribute to an unstable regulation of heart rate and thus precipitate the occurrence of cardiac rhythm and/or blood pressure regulation abnormalities (Shi et al., 2000; Wray et al., 2001). Furthermore, delays in baroreflex latency may compromise rapid reflex adjustment to cardiovascular stressors. Taking this into account, we speculate that in the presence of an increased baroreflex latency, the effector response (i.e. chronotropic response) may fall into the next oscillation of the input variable (i.e. blood pressure), when a different response would be required to buffer it. If endured over enough cycles, the result is a loss of adequate blood pressure control, leading to syncope episodes.

Other ANS reflexes may also be involved and dysfunctional in these patients. Regardless, the question remains: where and how these autonomic pathways are involved in vasovagal syncope? Further insight into the afferent and central integrative regions function in vasovagal syncope patients may prove fruitful to elucidate their mechanism.

F. Can the result of a head-up tilt table test be predicted?

Our results show that younger female patients and patients with lower BEI (<54.8%), in addition to the higher delay of the baroreceptor response (> 1.5 seconds) and a higher initial LF_{SBP} power, are more likely to have positive HUT table response. Furthermore, the 5 minutes BEI values maintained its predictive value when considered separately; however, risk stratification was even more effective when integrated with the other variables that have shown to play independent roles in predicting head-up tilt test outcomes. These results strengthen the hypothesis that impaired baroreflex function may represent a pathophysiological marker of altered response to orthostatic stress and may play a role in the pathophysiology of vasovagal syncope.

Our results are in line with some of the available literature. The number of previous episodes, age and female gender, are known independent predictors of syncope recurrence (Sheldon et al., 1996; Sheldon, Sexton, & Koshman, 2000; Grimm et al., 1997; Malik et al., 1997; Pitzalis et al., 2003; Aydin et al., 2009). Iacoviello et al. after following 190 patients for up to 18 months, during which 34 experienced a total of 90 episodes of syncope recurrence, has shown that female gender and BRS below median value after the start of HUT were significantly associated with the recurrence of syncope. Differently from us, these authors did not investigate these parameters as a forecasting tool for the HUT test outcomes.

Different approaches have been applied in order to predict HUT outcomes. Bellard et al. showed that transthoracic impedance could detect differences in central haemodynamics between fainters and non-fainters during supine rest and the initial period of 70^{o} HUT, with a consistent sensitivity and specificity, when combined with peripheral haemodynamic variables (Bellard et al., 2003). Hausenloy and colleagues studied the oscillations of sBP during the HUT test. They reported that the presence of haemodynamic instability, as indicated by oscillations in blood pressure, such as a sBP variation >30 mmHg, strongly predicts a positive HUT test with a sensitivity of 88% and positive predictive accuracy of 87% for subsequent vasovagal syncope (Hausenloy et al., 2009). Pitzalis et al. observed that drops of sBP during the first 15 minutes of HUT can

be used for the prediction of its outcome. In these authors' study, more than 14 asymptomatic reductions of systolic arterial pressure during the first 15 minutes of tilt allowed them to predict a positive test with 93 % sensitivity, 58 % specificity, and positive and negative predictive values of 28 and 98 %, respectively (Pitzalis et al., 2002).

Far less is known regarding the roles of the ANS and baroreflex function in predicting syncope. Approaches using heart rate and blood pressure variabilities alone seem to have limited predictive value, with prediction of syncope requiring the use of multiparametric analysis tools (Pruvot, Vesin, Schlaepfer, Eromer, & Kappenberger, 1994; Kochiadakis et al., 1997; Furlan et al., 1998; Pitzalis et al., 2002). Virag et al. developed an algorithm using ECG and blood pressure, to provide warning of an impending episode of vasovagal syncope (Virag, Sutton, Vetter, Markowitz, & Erickson, 2007). In 1380 consecutive patients, simultaneous analysis of R-R interval and sBP trends during HUT, as well as their variability, were performed in other to generate a cumulative risk that was compared with a predetermined vasovagal risk threshold. Using this approach, vasovagal syncope was predicted in 719 of 759 patients (sensitivity 95%), whereas 29 false alarms were generated in 396 tilt-negative patients (specificity 93%). In a follow-up study, the same authors applied their algorithm prospectively to 140 patients with suspected vasovagal syncope. The algorithm performance in predicting syncope recurrence using simultaneous heart rate and systolic blood pressure measurements, had a high sensitivity of 97.6% and specificity of 88.2%, with a median prediction time of 1 minute and 25 seconds (Virag et al., 2018).

Notwithstanding, other researchers have reported negative results on predicting syncope. Several other authors have not observed significant differences in the heart rate variability parameters during the first few minutes of the test between those with a HUT-positive and those with a negative outcome (Ruiz, Madoery, Arnaldo, Menéndez, & Tentori, 2000; Duplyakov, Golovina, Sysuenkova, & Garkina, 2011; Klemenc & Štrumbelj, 2015).

The reproducibility of our predictive model needs further validation. If results are confirmed in a broader population, several implications may arise. The first is to avoid

syncope in the laboratory, allowing for the termination of the test before the patient experiences syncope. The second is the implementation of this model in an external or implantable device (Brignole, Sutton, et al., 2006; Somlói, Toldy-Schedel, Nényei, Böszörményi, & Tomcsányi, 2015), providing patients with continued monitoring and timely diagnosis, leading to a third implication, which is patients education, allowing them to recognize an impending syncope, enabling adoption of corrective measures or allowing for a more effective tilt training protocol.

Conclusions

In summary, our results demonstrate dysregulation of the autonomic and baroreceptor reflex behaviour in recurrent syncope patients (regardless of age and sex), upon an orthostatic stress test. Furthermore, this dysfunction may be the primary mechanism underlying head-up tilt induced syncope for the aforementioned population. Overall, the present findings proved to be a reliable clinical tool to stratify reflex syncope patients in order to define an integrated and personalised therapeutic scheme. Results from this study will be integrated into the development of predictive tools, which can be used by both clinicians and patients as complementary measures to improve symptoms.

Chapter 6

CHAPTER 6

THE MANAGEMENT OF REFLEX SYNCOPE

Exploring the hypothesis under study…

The management of reflex syncope is multifactorial and complex. It involves several types of treatment strategies due to the multiple pathophysiological mechanisms that have been described. They involve patient education, including the avoidance of putative syncopal stimulus, the usage of physical counter-pressure measures, the increase of water and salt ingestion, drug treatments with beta-blockers, alfa-adrenergic drugs, and steroids, among others, and the autonomic modulation. Regrettably, there are no widely agreed-on specific treatments, and the benefits of drug therapy are often disappointing. Autonomic modulation through orthostatic training may be an effective way of syncope management but, up to the moment, the available results do not support its wide acceptance.

Thus, in accordance, as a working hypothesis, we establish that:

A program of orthostatic training, performed under a personalised and multimodal protocol is useful in the management of reflex syncope

I. Introduction

Vasovagal syncope is the most common form of syncope, with a higher incidence in young adults (Ganzeboom et al., 2003; Wieling, Ganzeboom, & Saul, 2004a). Its pathophysiology is poorly understood but, as we have previously demonstrated, alterations in the reactions of the autonomic nervous system and baroreflex control to stressful stimuli, such as standing or acute pain, are involved in triggering syncope. Various treatment options have been proposed over the years, ranging from behaviour

modification to cardiac pacing and multiple types of drug therapy, but none has been shown to be fully capable (Shen et al., 2017a; Brignole et al., 2018).

Tilt training, a non-pharmacological treatment modality for vasovagal syncope, was proposed in 1998 by Ector et al. (Ector et al., 1998) who showed that repeated and prolonged exposure of the cardiovascular system to orthostatic stimuli had therapeutic effects in patients with syncope. The effectiveness of tilt training has been evaluated in several subsequent studies (Di Girolamo, Di Iorio, Leonzio, Sabatini, & Barsotti, 1999; Abe, Kondo, Kohshi, & Nakashima, 2002; Foglia-Manzillo et al., 2004; Gajek, Zyśko, & Mazurek, 2006; Gardenghi et al., 2007; On et al., 2007; Duygu et al., 2008; Zeng, Ge, Zhang, Wang, & Guo, 2008; Laranjo et al., 2012), but the results have been inconsistent and contradictory, probably due to differences in patients' characteristics, training protocol and, particularly, patient compliance.

As a result, the mechanisms of action of tilt training are still poorly understood. Generally, it is thought that desensitisation of cardiopulmonary receptors to orthostatism may be involved, as well as autonomic remodelling and alterations in baroreflex activity. Thus, in the present study, within a reflex syncope group of patients refractory to the conventional measures, we sought to clarify the physiological mechanisms underlying tilt training, to assess the clinical outcomes of a tilt training programme on the recurrence of symptoms and quality of life and to identify surrogate markers to predict tilt training response in patients with vasovagal syncope.

II. Methods

A. Inclusion and Exclusion Criteria

We enrolled 102 patients in this study, all of them previously diagnosed with orthostatic induced reflex syncope, refractory to conventional measures (education on avoiding triggers, hydrosaline reinforcement, counterpressure manoeuvres, compression stockings) and drug therapy (midodrine and fludrocortisone), with head-up tilt test induced syncope, performed according to our centre's standard protocol.

All patients met the following inclusion criteria: 1) at least two syncopal episodes within the last six months or one syncope and three pre-syncopal episodes/ 6 months (defined as a transient alteration of consciousness, without complete loss); 2) absence of known structural and/or electrical heart disease; 3) absence of other evident aetiologies for syncope; and, 4) absence of criteria for orthostatic hypotension, defined as a sustained reduction of systolic blood pressure of at least 20 mm Hg or diastolic blood pressure of 10 mm Hg within 3 minutes of standing or head-up tilt to at least 60° on a tilt table. The following exclusion criteria were applied: (1) infrequent symptoms; (2) structural heart disease or cardiac rhythm disturbance; (3) neurologically mediated syncope including classical or delayed orthostatic hypotension; (4) excessive reaction to nitrates; (5) refusal to consent to the test; (6) physical inability to remain standing for 30 minutes; (7) impossibility of suspending cardiovascular medication that could interfere with the assessment of the autonomic nervous system; and (8) pregnancy.

The study, complying with the Declaration of Helsinki and the Oviedo Convention, was approved by the *Centro Hospitalar e Universitário de Lisboa Central* and the Faculty of Medicine of the University of Lisbon Ethics Committees and performed under written, informed consent from all participants.

B. Experimental Protocol

Tilt test protocol

Head-up tilt table test (HUT) was performed in a dedicated laboratory, characterised by a quiet environment with controlled temperature and humidity, during the morning period, after a light breakfast, without ingestion of caffeine, chocolate or other xanthines. No intravenous lines were used. Subjects were placed on a tilt table and, after a resting period of 10 minutes in the supine position, were tilted-up to a level of 70 degrees, at a constant speed, for 15 seconds. Subjects were instructed to breathe normally. If no spontaneous syncope had occurred after 20 minutes at the 70 degrees orthostatic position, 375µg sublingual nitrates were administered. The test was terminated either by a positive response or 20 minutes after nitrate administration. As soon as symptoms (syncope or pre-syncope) developed during the adaptation period, the table was lowered to the horizontal position, and the test was terminated. Following the return to the supine position, a second resting period of 5 minutes was allowed to elapse to guarantee a stable condition. A positive response to HUT was defined as a sudden development of syncope or pre-syncope associated with hypotension, bradycardia, or both, while a negative test was determined as the absence of hypotension, bradycardia, syncope, or pre-syncope. Positive responses were classified following the VASIS classification.

Tilt Training Programme

The tilt training programme had two simultaneous components. The first part consisted of nine in-hospital tilt training sessions, three times a week, during the morning period, after a light breakfast, without ingestion of caffeine or other xanthines. Each session had a 30 minutes duration. Training took place in a laboratory designed for autonomic evaluation, in a calm environment and with controlled temperature and humidity. Patients were recommended to breathe normally during the duration of the sessions, the breathing rate and the amplitude being controlled through a respiratory belt. After 15 minutes of supine (0^o) rest, the patient was placed on a tilt table with feet supported and with restraining straps to prevent falls in the event of syncope. For the first two weeks the tilt table was at 60^o, and at 70^o for the third week. During training, the patient

underwent continuous non-invasive monitoring of blood pressure (BP), electrocardiogram (ECG) and thoracic impedance (Task Force Monitor, CNSystems, Austria), and the test was stopped if symptoms occurred. Finally, hospital sessions were complemented by daily home training, which corresponds to the second part of the programme. Patients were instructed to stand in orthostatic position at 60^{o} against a flat vertical surface with feet apart about 15 cm from the wall for 20 minutes and to sleep at ten degrees head-up. Patients were instructed to stop the daily orthostatic manoeuvre whenever they felt the onset of symptoms. Patients were told to do some activity during the training period (listening to music, watching television, reading a book), to promote compliance with the daily home-training. All participants were expected to fill out a diary, with the relevant data of home training, including date, session duration, and any symptoms that might have occurred.

C. Data Acquisition and Analysis

Beat-to-beat RR-intervals (RRI) and systolic arterial blood pressure (sBP) were continuously recorded. The non-invasive beat-to-beat recording of stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were computed using the data from the cardio-thoracic impedance signal.

The raw signal was filtered to remove movement and electrical noise. The signal was further filtered using a polynomial trend removal algorithm and a bandpass filter to isolate the periodicities of interest and minimise the effects of non-stationarities. A continuous visual inspection was performed in order to ensure that only data that did not contain any abnormal beats was extracted. A peak-to-peak routine was implemented to detect sBP peaks and the fiducial points of the R waves of each ECG complex, in order to construct a time curve of sBP (systogram) and HR (tachogram). A cubic spline interpolated the tacho and systogram, which were resampled (resampling period =0.193 s). The reconstructed signals were used to calculate HR and sBP variability and baroreflex sensitivity.

All data analysis was performed using our in-house computational interface - FisioSinal. As recommended, the total manoeuvre time of each tilt-training session was divided into 5-minute epochs (Malik et al., 1996), the first being the baseline period and the others on the tilt table, to facilitate computation and to better visualise the dynamics of cardiovascular alterations taking place during the session.

1) Heart rate and blood pressure variability

Heart rate and sBP variability were assessed through the modified Hilbert-Huang transform (mHHT) (please refer to *Chapter 4* for further details). The mHHT is adaptively data-driven and possesses the higher time and frequency resolution than other frequency and time-frequency methods (Huang et al., 1998; Huang, 2005a; Huang & Wu, 2008; Tavares et al., 2012). Briefly, this method first decomposes the signal into a finite and often small number of functions (empirical mode decomposition – EMD), the intrinsic mode functions (IMF's). The IMFs are produced sequentially with each subsequent IMF being derived from the initial residual signal. The iterative algorithm is called sifting. Then the Hilbert transform is performed and yields the instantaneous frequency, phase and amplitude of the signal. The selected intrinsic mode functions relate to signal frequencies between 0.038–0.15 Hz (LF) and 0.15–0.6 Hz (HF) (Malik et al., 1996)). The square of the selected intrinsic mode functions was then calculated and, for each interval of time, LF and HF variability bands were computed as the integrals of the IMF's associated with the frequency ranges of interest.

2) Baroreflex sensitivity

Baroreflex sensitivity was computed using the sequence method as stated elsewhere (Parati et al., 2000; Di Rienzo, Parati, et al., 2001a) (please refer to *Chapter 4* and *Chapter 5* for further details). The sequence method is based on the spontaneous presence of concurrent RRI and sBP changes (increase or decrease) over at least three consecutive heartbeats (sequence). In brief, this estimation was based on the analysis of beat-to-beat series of systolic blood pressure scanned to identify ramps of 3 or more consecutive heartbeats with a progressive increase ("up-ramp") or decrease ("down-ramp") of at least 1 mmHg, regardless of the possible occurrence of concomitant RR interval changes. The algorithm identifies spontaneous sequences, defined as systolic blood pressure

ramps, followed by concomitant and concordant RR intervals variations of 5 milliseconds coupled with 0-, 1-, and 2-beat lags, with each sequence being included only once. For each sequence, the slope of the linear interrelationship between sBP and the following RR intervals values was calculated and was considered reliable when the correlation coefficient (r) was higher than 0.80. For each period of analysis, the baroreflex effectiveness index (BEI) was defined as the ratio between the total number of baroreflex sequences detected and the total number of sBP ramp-like changes in blood pressure, regardless of whether the latter is followed by a change in RR-interval or not. The higher the effectiveness value, the more systolic blood pressure ramp changes are followed by a change in RR-interval.

3) Cross-Spectral Wavelet Coherence and Phase

The coherence is a complex function of the frequency and estimates a degree of linear correlation between two signals. High coherence suggests the capability of one time series to predict the following one. Availability of the cross-spectrum also allows for the calculation of the phase relationship in the range of -180º and +180º, being represented as the phase spectrum.

In this study, Cross Spectral Wavelet Coherence analysis was performed in a Matlab environment (Matlab 2017b, MathWorks, MA, USA) using an in-lab developed solution, based on the seminal works of Torrence and Compo (Torrence & Compo, 1998) and Grinsted and co-workers (Grinsted et al., 2004). In brief (Yang et al., 2008; Laranjo et al., 2014), a complex wavelet transform (Morlet wavelet) was used for feature extraction from cardiovascular signals analysed for intervals of 5 minutes. Continuous wavelet transform (CWT) scalograms were calculated for the sBP and the heart period variables. From the two CWTs, a Cross Wavelet Transform (CrWT) was constructed, exposing the common power and relative phase in time-frequency space. Next, the Wavelet Coherence (WTC) between the two CWT was computed. The coherence spectrum, presenting values between 0 and 1, is a measure of the correlation between the variations of the two signals for a given frequency. The phase spectrum shows, at each frequency, the phase difference (lead or lag) between the signals, from where the lag period (in seconds) between the two time series was computed.

D. Outcomes Assessment

Clinical Efficacy

Following the tilt training programme, patients were classified into two categories, based on the absence of syncope and a significant decrease in pre-syncope episodes, or the recurrence of syncope, with or without a significant change in pre-syncope episodes: responders and non-responders, respectively. All patients have been followed-up clinically at one month and every six months, for a maximum of 48 months. Furthermore, to the non-responders was offered a second series of nine sessions, following the above-depicted protocol. Also, patients were contacted by telephone once a month (for the first six months) and then yearly (after the 1st year). Clinical efficacy was assessed by the number of spontaneous syncopal and presyncope episodes during the follow-up period, compared with the number of syncopal and presyncope episodes that occurred during the last 12 months before the tilt training programme.

Quality of Life Assessment

In order to assess the impact of the tilt training protocol in patients' quality of life (QoL), a subgroup from the total tilt training population, consisting of 20 consecutive patients with recurrent reflex syncope, was assessed with the "Impact of Syncope on Quality of Life" (ISQL) scale, validated to the Portuguese population (Nave-Leal et al., 2015). Their QoL was assessed at two different time moments: before starting the programme and at 12 months of follow-up. Their results were compared with the ones from two different groups: a group of reflex syncope patients (n=20) matching gender and age, who were treated with general anti-syncope measures (education on avoiding triggers, hydrosaline reinforcement, compression stockings) plus counterpressure manoeuvres – the general measures & counterpressure manoeuvres group - and a control group of reflex syncope patients (n=20) to whom only general anti-syncope measures were explained, with no other structured approach being taught (the 'no-intervention' group).

E. Statistical analysis

Continuous variables are expressed as mean and standard deviation and plotted as the composite of the mean values of all subjects unless otherwise specified. Categorical variables are reported as frequencies and percentage of patients. Normality distribution of the continuous variables was analysed with the Kolmogorov-Smirnov test (Lilliefors' correction), and the Levene's test was used for assessment of homogeneity of variance. Data from each tilt training sessions are represented along the time. Student's t-test for paired data was used to compare the results of the first and ninth tilt-training sessions. A Spearman's correlation was run to determine the relationship between the clinical characteristics of the patients (i.e., total number or type of syncope and pre-syncope episodes in the pre-tilt training) with the attained results in the post-tilt training phase. Probability of syncope free survival (SFS) after finishing the tilt training programme was calculated from the day of the ninth tilt training session until the first recurrence or last follow-up. The survival estimates were calculated using the Kaplan–Meier method. The differences in SFS between genders were compared using the log-rank test. Receiver operating characteristics (ROC) curves were constructed to assess the power of the autonomic and baroreflex function indices at the ninth tilt training session in predicting syncope recurrence, and the area under the curve (AUC) was calculated. A value of $p<0.05$ was considered statistically significant. Data were analysed using SPSS software version 23 (IBM Corporation, USA).

III. Results

One hundred and two patients were included in this study (57,8% female gender), with a mean age of 46±18 years (range 16-82 years-old). The population had a slightly asymmetric right-skewed distribution (Figure 27). Thirty-six patients (35,2%) had cardioinhibitory reflex syncope, 34 patients (33,3%) mixed-type and 32 patients (31,37%) vasodepressor-type reflex syncope. The mean number of syncopal episodes before starting the tilt training programme was of 6±3 syncopes/patient/year with 11±6 pre-syncopes /patient/year in the 12 months before being included in the tilt training programme.

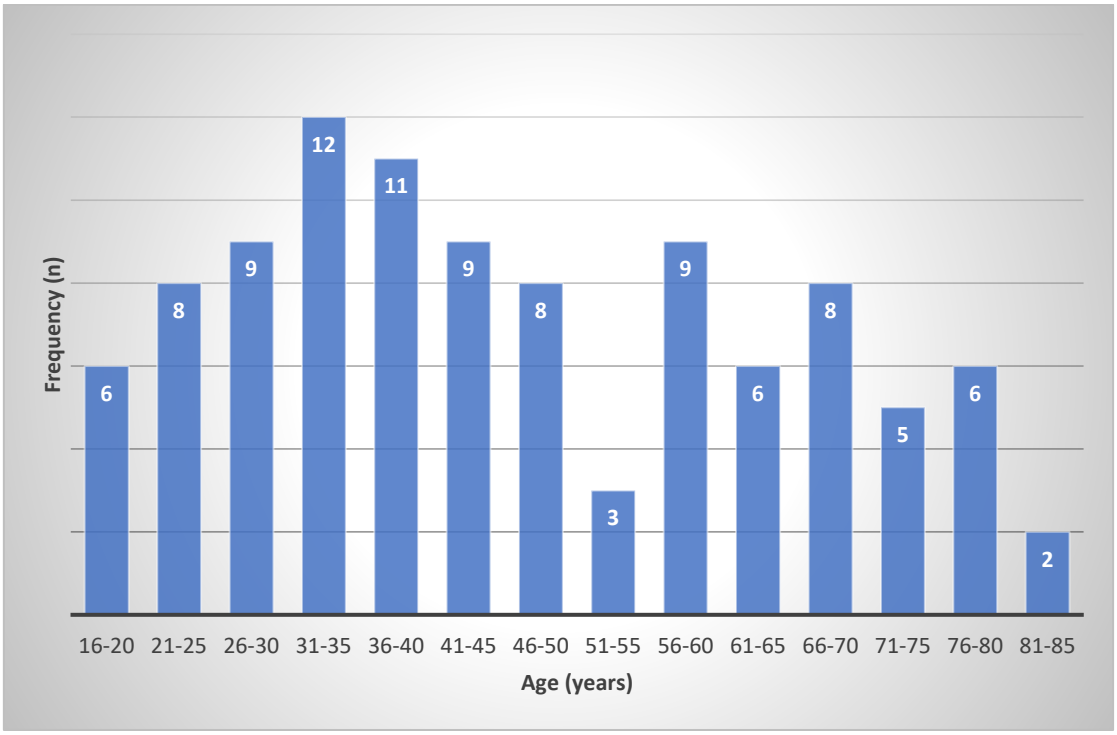


Figure 27. Age distribution of the patients enrolled in the tilt-training programme.

A. Follow-up and clinical efficacy

The average time of follow-up was of 37.7±11.2 months (range 12-48 months), with all patients having completed, at least, 12 months of follow-up and 47 patients (46%) having completed 48 months of follow-up. There were no dropouts during the follow-up period. Although all patients completed the in-hospital sessions, after one year, 25 of them (24%) interrupted home self-training due to symptomatic improvement, with a total of 39 patients (38%) having ceased the home self-training at some point during follow-up.

After tilt training, most of the patients did not present other episodes of syncope ('responders') (n=89; 86,3%) or pre-syncope (n=82, 80%). In eight patients there was a significant decrease in the number of episodes of syncope (from 6±3/patient/year in the 12 months before tilt training to 1±1/patient/year post-tilt training, p=0.0008), as well as pre-syncope (from 12 ±6/patient/year in the 12 months before tilt training to 5±3/patient/year during follow-up, p=0.0019). The majority of these patients had the first recurrence of symptoms at around the 5th month (range 1-15 months) after

finishing the in-hospital protocol (Figure 28), and most of them (n=9) had ceased the home self-training before the recurrence of symptoms (range 1-9 months before the 1st recurrence). In the remaining five patients, no change in the pattern of episodes was seen after the first series of nine tilt training sessions. These two last groups of patients (n=13, 12.7%) comprise the 'non-responders' group to the tilt training programme. The clinical characteristics of 'responders' and 'non-responders' are depicted in Table 11.

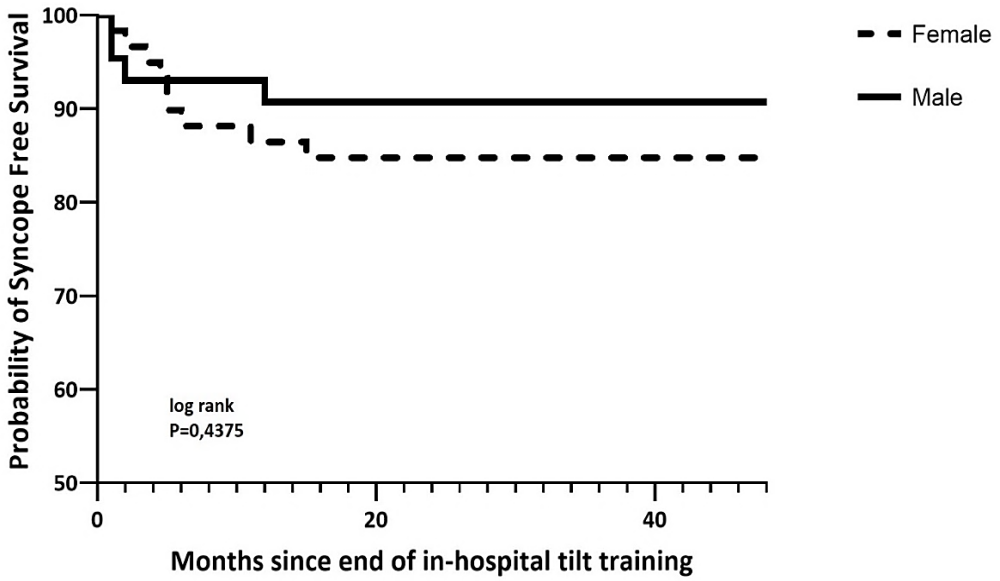


Figure 28. Probability of recurrence of Syncope in the TILT Training Population.
Time to first syncopal recurrence, according to gender, during follow-up. There was no significant difference between genders (p = 0.437).

Table 11. Clinical characteristics of responders and non- responders

	Responders (n=89)	Non-Responders (n=13)	p
Female gender	51 (57,3%)	8 (61.5%)	0.72
Age (years)	45±14,7	48,13±27.3	0.55
Syncopal episodes in the last 12 months	6±3.5/patient/year	6.4±3.2/patient/year	0.63
Pre-syncopal episodes in the last 12 months	10,7±8,1 /patient/year	12.1 ± 5.6/patient/year	0.55
Syncope type	Cardioinhibitory (n=32; 35,9%) Mixed (n=29; 32.5%) Vasodepressor (n=28; 31,4%).	Cardioinhibitory (n=4; 30,7%) Mixed (n=5; 38.4%) Vasodepressor (n=4; 30.7%).	0.71

Non-responders tended to be older and to be more symptomatic. Despite that, there was no relation between the total number or type of syncope in the pre-tilt training with the attained results in the post-tilt training phase as Spearman Correlation Coefficient showed a weak negative association between these variables (Spearman coefficient r = -0.034), which is not statistically significant.

B. Quality of Life Assessment

The clinical characteristics of the cohorts studied in the QoL assessment, 'tilt training', 'general measures plus counterpressure manoeuvres' and 'no structured intervention' are depicted in Table 12. Patients were age and sex-matched, and no significant difference was found in the frequency of symptoms at enrolment or regarding the type of syncope at the diagnostic head-up tilt test.

Table 12. Clinical characteristics of patients enrolled in the QoL sub-analysis

	Tilt training (n=20)	General measure & counterpressure (n=20)	No-intervention (n=20)	p
Female gender	13 (65%)	11 (61.5%)	13 (65%)	0.76
Age (years)	51.5 ± 20.6	47,2± 19.5	52.7±22.9	0.68
Syncopal episodes in the last 12 months	6.2±4,2/pt/year	5.1±3.9/pt/year	5.7±2.4/pt/year	0.62
Pre-syncopal episodes in the last 12 months	9.7±6.1 /pt/year	8.4 ± 3.3/pt/year	9.1±4.8 /pt/year	0.70

In the tilt training QoL cohort, 20 patients (13 females, 65%) were included. They had a mean age of 51.5 ± 20.6 years (range 18-78), the majority was married (65.2%) and in active duty (60%). Eight patients (40%) had vasodepressor, 7 patients (35%) cardioinhibitory and 5 patients (25%) mixed-type reflex syncope. Before being enrolled in the tilt training programme, they had syncopal episodes for an average 9±10,6 years (range 1-24 years). In the 12 months before tilt training, the mean number of syncopal episodes was of 6±4/patient with 10±6 pre-syncope/patient. One year after the tilt training programme, 17 patients (85%) were symptom-free. The remaining three

patients had no syncopal episodes but reported an average of 3±3 pre-syncope episodes since ending the in-hospital tilt training programme, which constitutes a reduction of almost two thirds, when compared with the basal frequency.

In the general measures plus counterpressure manoeuvres group, four patients (20%) were symptom free at 6 months follow-up and, in the remaining patients, a decrease on the frequency of syncope and pre-syncope episodes were documented (4±3 vs 5±4/patient/year, p=0.19 and 6±4 vs 8±3/patient/year, p=0.09, respectively). No changes were found in the non-structured intervention group, with all patients presenting an average of 5±4 syncope episodes/patient/year (p=0.55).

In this population, the tilt training responders' group (n=17) obtained sustained benefits in QoL, as measured by the ISQL scale. At the 12th months' follow-up, the tilt training programme was associated with a QoL improvement in the ISQL items related to worry, fear and frustration with the difficulties experienced (Table 13). Results from other evaluation measures did not present statistically significant alterations, but a global trend for improvement could be demonstrated. In the general measures plus counterpressure manoeuvres group, only the item of "misunderstanding by others of the impact of their clinical condition" was significantly improved, and no changes of the reported QoL were documented in the no structured-interventions group.

Table 13. Improvement on QoL in the tilt training responders' group

	Enrolment	12 months FUP	P-value
As a result of your fainting or lightheaded spells, how often in the last month have you			
Felt tired and worn out?	3,8±1.7	4.7±1.4	0.122
Felt frustrated?	4.7±1.3	4.8±1.6	0.364
Been limited in the type of work you could do?	4.4±1.8	5.1±1.3	0.134
Been worried about fainting?	3.9±1.7	5.3±1	**0.004**
Been frightened of fainting?	3.9±1.7	5.1±1.4	**0.007**
Felt that fainting and lightheaded spells have interfered with performing vigorous physical activity?	3,8±2	4.9±1.8	**0.027**
Think back over the last month and indicate how much you agree with the following statements			
Because of my fainting, I accomplish less than I would like to	2.5±1.8	3.4±1.7	**0.031**
No one understands the effect that fainting has on my life	2.8±1.5	3.1±1.8	0.230
My fainting makes me feel confused	2.1±1.4	2.8±1.9	0.136
Think back over the last month and indicate how often you have avoided			
Driving a vehicle	3,0±1.7	4.1±1.4	0.107
Standing for long periods of time (more than 5 min)	4,6±1.8	3.9±2.1	0.187
Being in warm or hot environments due to the fear of fainting	4±1.6	3.1±2	0.112

Score: 1-Always; 2- Almost always; 3- Most of the times; 4 – Sometimes; 5 – Few times; 6 – Never

C. Physiological Adaptations to the Tilt Training Programme

Hemodynamic Parameters

The chronotropic profile suffered changes between the first and the ninth tilt training sessions (Table 22 from Appendix 1). While in the first session, a marked and significant tachycardia was observed upon tilt and during the remainder of the manoeuvre, by the ninth session, a steady, progressive and non-significant sinus tachycardia was observed, lasting until the end of the manoeuvre. Besides, mean sBP, stroke volume and cardiac output showed a significant increase between the first and the ninth sessions (Figure 29).

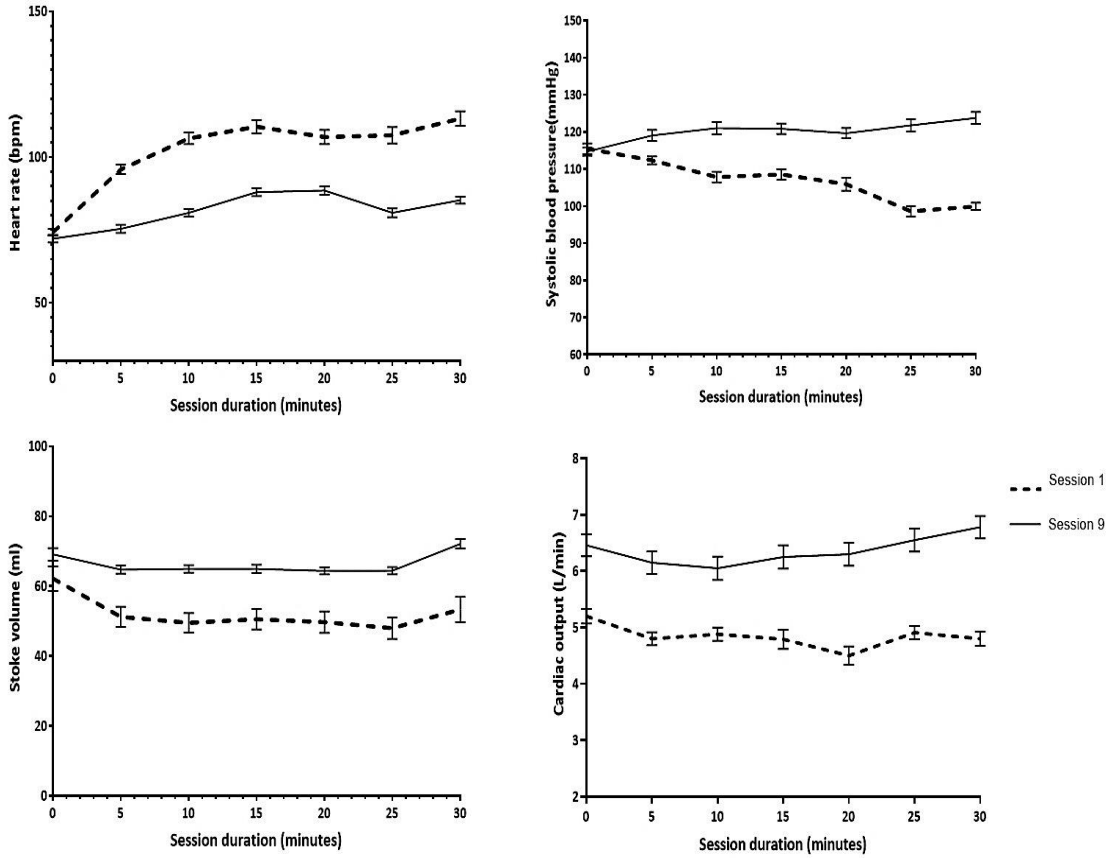


Figure 29. Evolution of haemodynamic markers during tilt training (Session 1 vs Session 9)

Figure 30 represents a statistically significant increase in total peripheral resistance (please refer to Table 22 for values), between the first and the ninth tilt training sessions, associated with a decrease in statistical dispersion measures, namely standard

deviation, reflecting a tendency to reach a steady-state on a new condition of lower dynamicity.

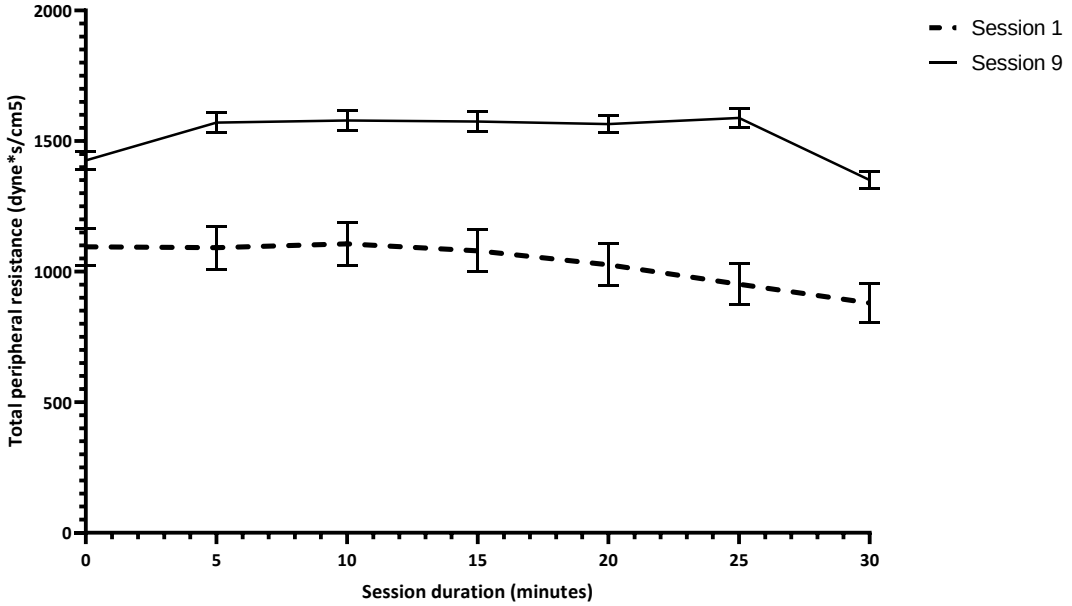


Figure 30. Evolution of total peripheral resistance during tilt training (Session 1 vs Session 9)

Cardiovascular autonomic function

In the assessment of cardiac autonomic function throughout the tilt training programme, analysis of heart rate and sBP variability using the mHHT showed a global increase in variability, translated by increases of the LF_{SBP} (sympathetic activity) and HF_{RRI} (parasympathetic activity) bands. Furthermore, a change in the pattern of the sympathetic nervous system response was seen from the first to the ninth tilt training session. In fact, in the first session, an initial sudden rise of sympathetic tone immediately after tilting-up, followed by a second overshoot of activity and continued by a steady fall-off could be documented. Throughout the tilt training program, this pattern was changed, being gradually replaced by a progressive increase in the LF_{SBP} power along time (Figure 31; values in Table 22, Table 23 in Appendix 1).

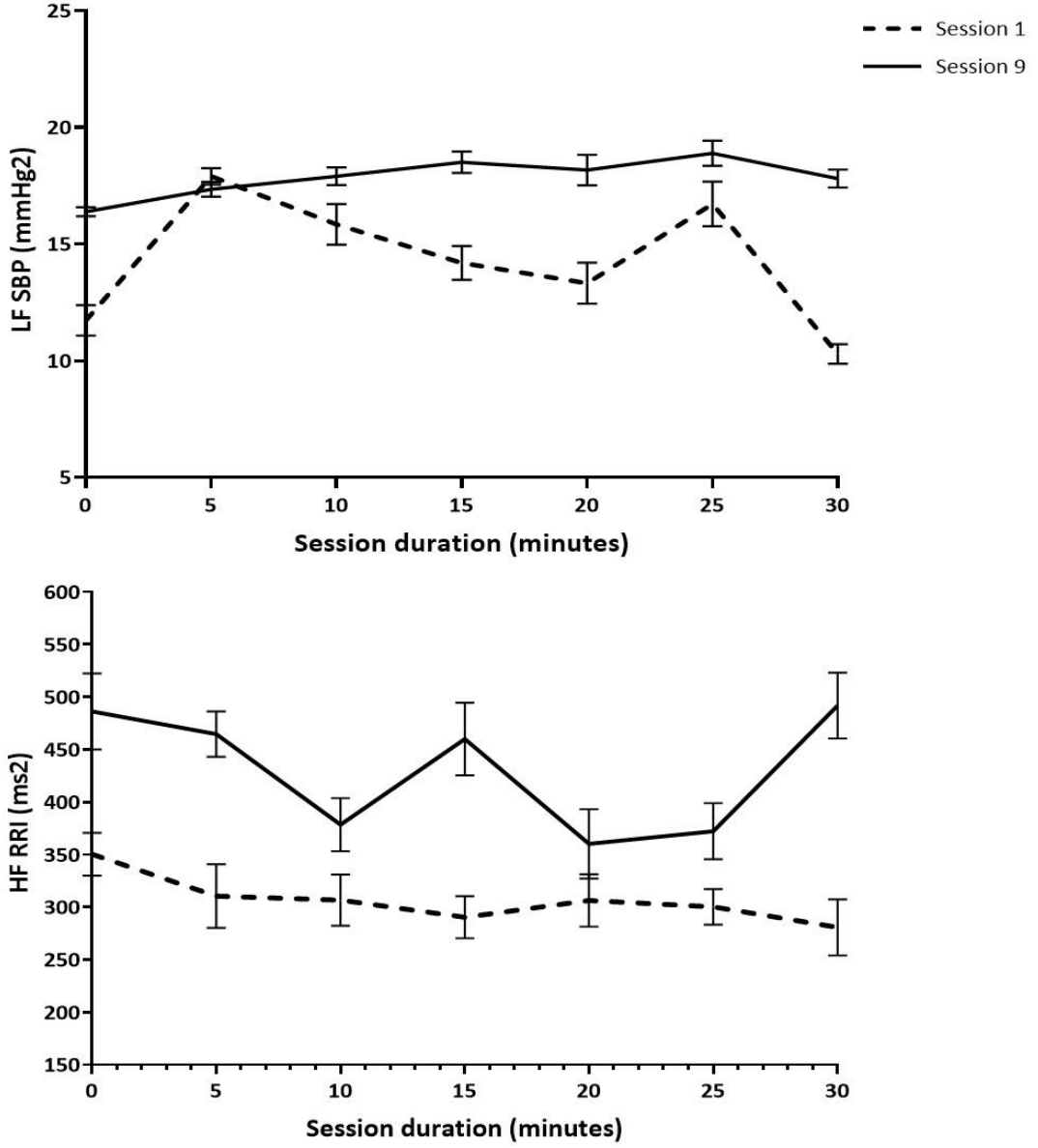


Figure 31. Evolution of heart and blood pressure variability during tilt training (Session 1 vs Session 9)

D. Baroreceptor reflex study

The evaluation of baroreflex sensitivity or gain (BRS) and its effectiveness (BEI) using the sequence method suggest a baroreceptor function resetting along the protocol (1st session vs. 9th session) with a significant increase in the number of systolic blood pressure ramps both in supine position (3.1±1.9 vs 4.8±1.5 sBP ramps /100 heart-beats; p=0.001) and after head-up tilt (3.5±2.1 vs. 6.5±2.7 sBP ramps /100 heart-beats; p<0.001). An increase in sequences was also observed towards the end of the training. Actually, at the time of the first tilt training session, baroreflex sequences represented

13.4±5.8% of the overall analysed sequences, while being of 18.7±4.1% (p=0.0001) by the ninth session.

In the supine position, no differences were found between the first and the ninth tilt training session, with patients having similar BEI's (55.81±26.01% vs 59.12±22.39%, p=NS). However, after head-up tilt, tilt training responders showed a different trend, with a significantly higher BEI value at the end of the tilt training programme (Figure 32). For instance, while in the first session patients showed a progressive albeit non-significant decrease of BEI (56.20±23.88% immediately after tilt vs 50.43±31.78% at the end of the first session; p=0.1442) towards the end of the training, responders showed a progressive increased BEI, till reaching a plateau, which was maintained throughout the last session.

Tilt-training "responders" could be differentiated from 'non-responders' based on BEI response pattern at the ninth tilt training session. Responders presented lower BEI values at the supine position, which increased after head-up tilt, while for non-responders, a statistically significant decrease could be observed during the manoeuvre (Figure 32). A BEI cut off value > 57.5 at the first five minutes of head-up tilt on the ninth tilt training session had an AUC of 0.9023 (p< 0.0001), with a sensitivity of 89.89% and specificity of 84.62% in predicting response to tilt training programme (Figure 33).

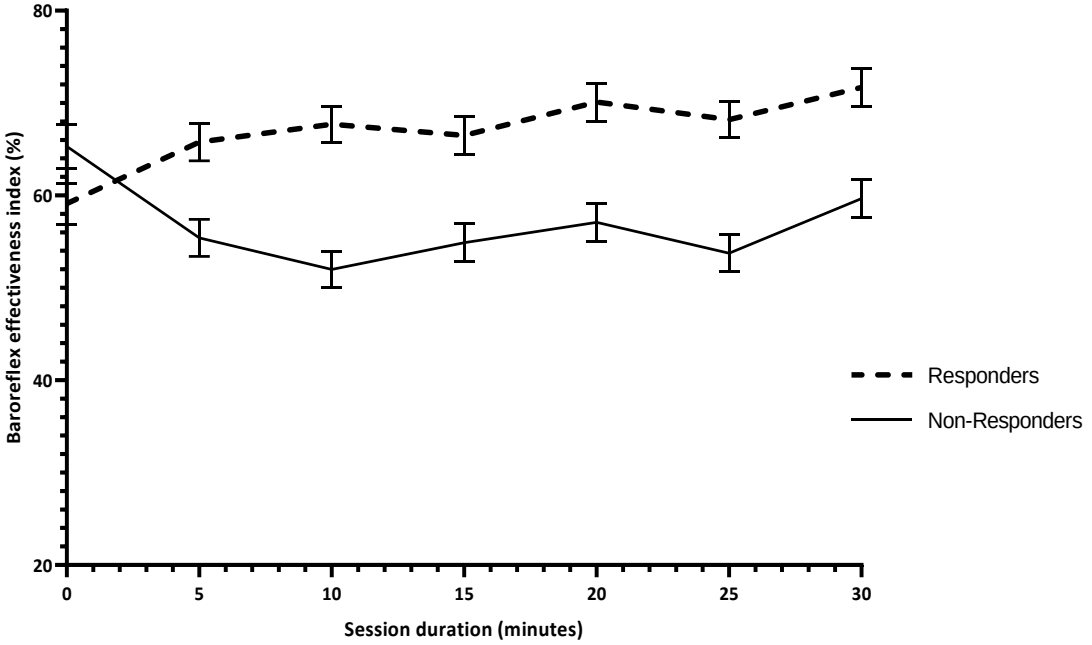


Figure 32. Evolution of Baroreflex Effectiveness Index (BEI) at the ninth tilt training (responders vs non-responders)

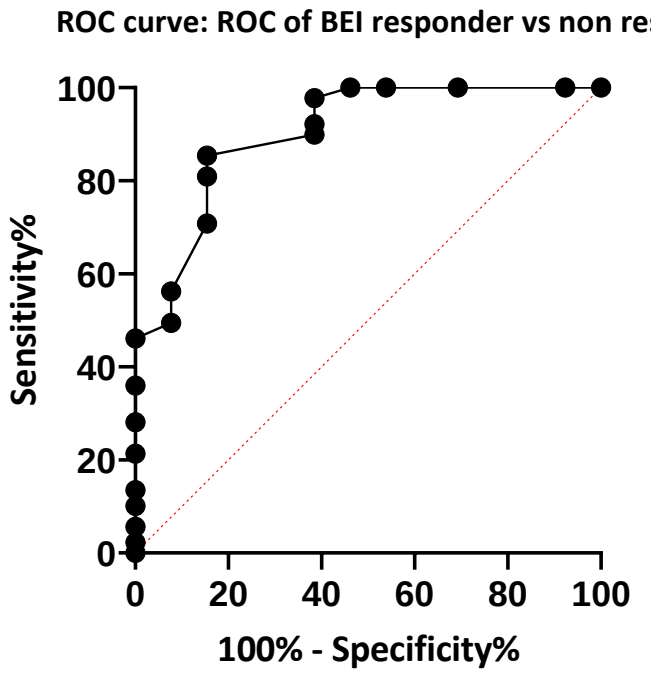


Figure 33. Baroreflex sensitivity in the first five minutes in a standing position (ROC analysis).
A BEI cut off value > 57.5 at the first five minutes of head-up tilt on the ninth tilt training session had an AUC 0.9023 (p< 0.0001) with a sensitivity of 89.89% and specificity of 84.62% in predicting response to tilt training programme.

E. Wavelet Coherence and Phase analysis

The responders' group showed a progressive increase of the LF and HF band coherence from the first to the ninth tilt training session. At the ninth session, the coherence pattern was similar to the head-up tilt test coherence of a healthy, tilt negative individual, as seen in Figure 34. Conversely, in the non-responder group, two patterns of response could be noticed: a) a complete lack of improvement of the heart rate and SBP variability coherence which is associated with an ineffective tilt training (Figure 35A) and b) a slower improvement of BRS, BEI and coherence throughout the training programme (Figure 35B).

The time delay in seconds, derived from the phase analysis, indicates that the responders increased their baroreflex performance. Systolic blood pressure and RR-intervals LF spectral bands oscillate with mean synchronicity of 1.8±0.24 seconds, at the first 5 minutes of orthostatic position during the first tilt training session which, at the ninth tilt training session, decreased to an average delay of 0.9±0.39 seconds, with a maximum delay under 1.1 seconds.

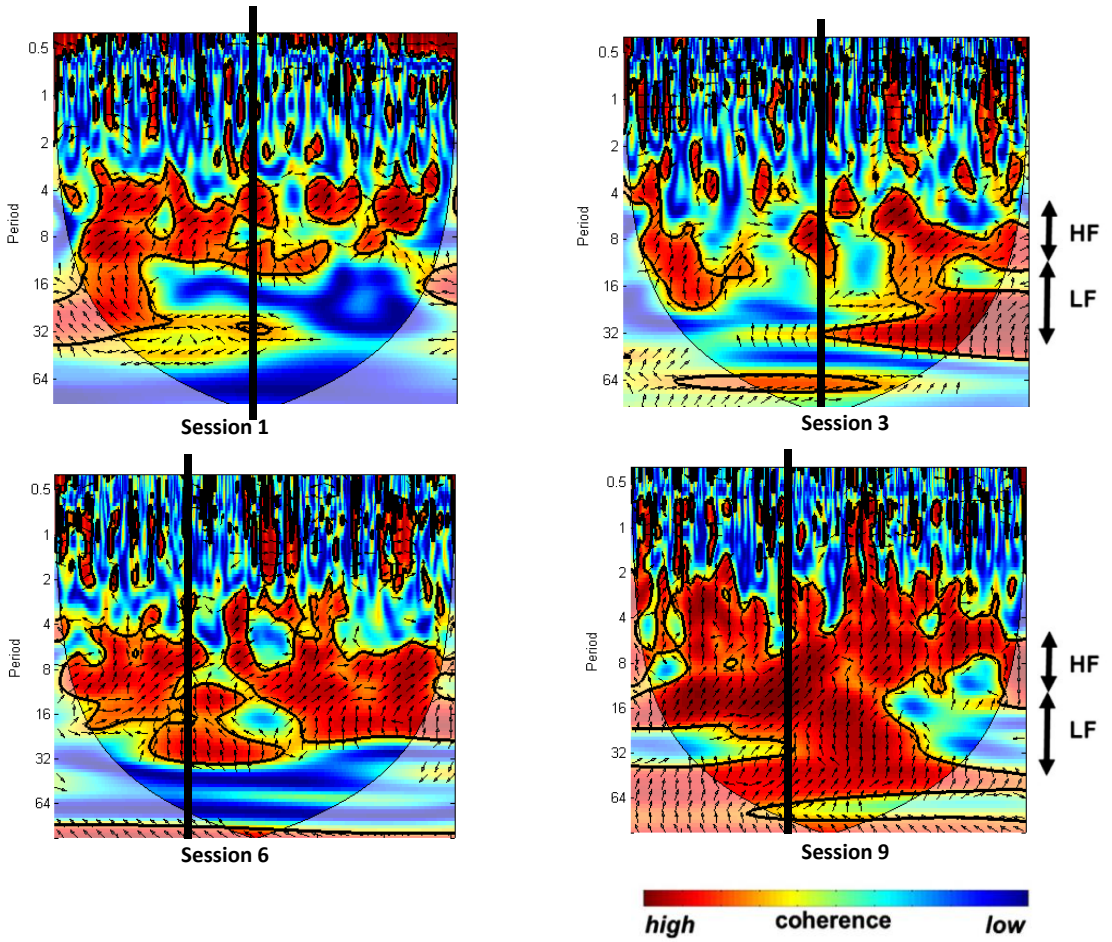


Figure 34. Modification of HR and SBP variability coherence along the training period in a responder patient.

The increase of coherence along the sessions, which relates with an increase of baroreceptor remodelling, is represented by an improvement of the band organization together with a higher density of the orange/red colour. These graphic changes are best seen after the tilting-up (vertical black line).

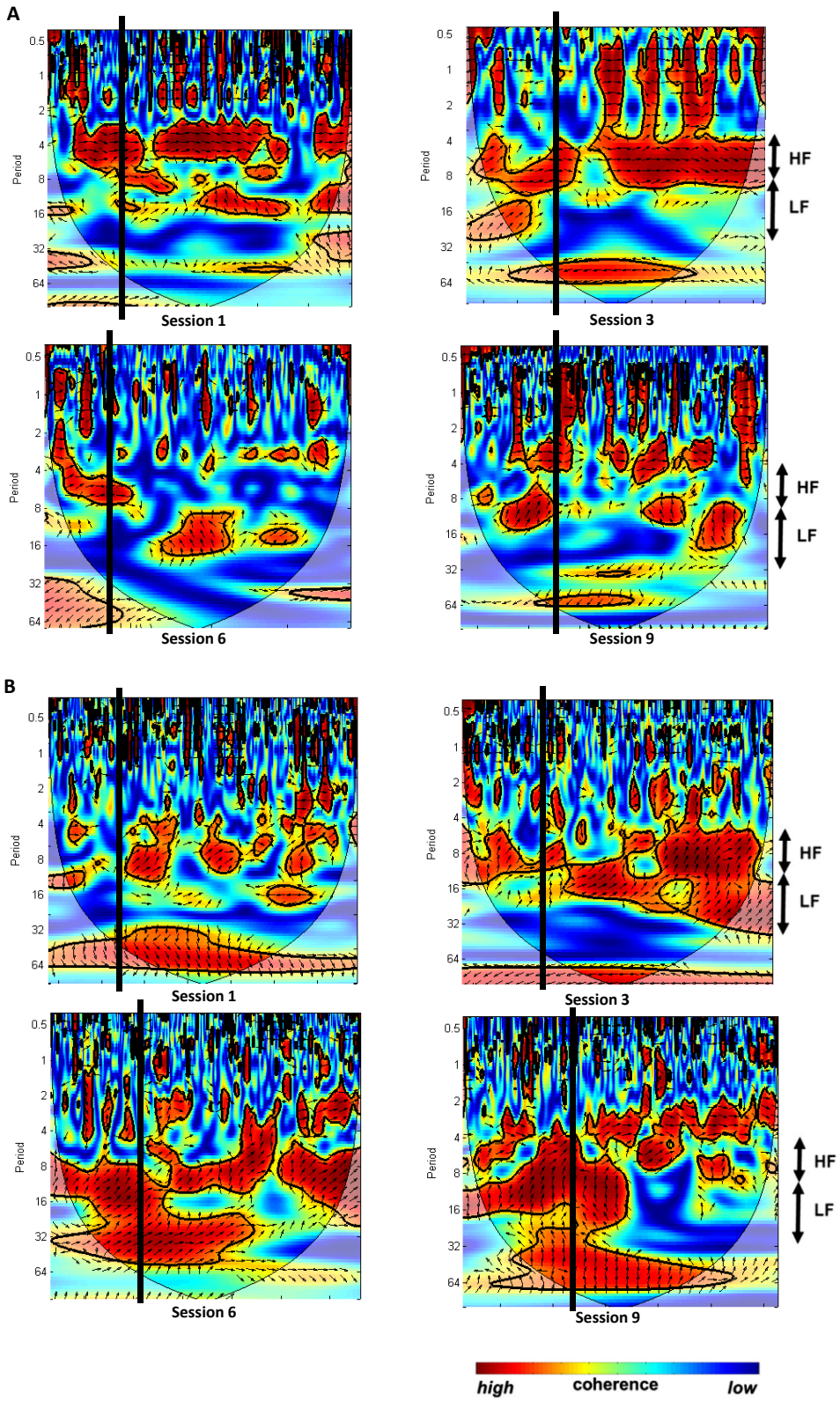


Figure 35. Modification of HR and SBP variability coherence along the training period in two non-respondent patients (A and B).

IV. Discussion

Reflex syncope is the most common cause of transient loss of consciousness. Although prognosis is good, it is associated with a marked reduction in QoL, due both to the risk of physical injury and loss of self-confidence induced by fear of further episodes. The clinical management of reflex syncope has a broad spectrum of therapeutic measures, whose efficacy has not been clearly established. In general, these measures are limited to improve patients' overall QoL by preventing new syncope episodes that may result in physical injury. In the last decades, many studies were published proposing various treatment modalities for reflex syncope, ranging from dietary measures to the implantation of pacemakers with distinct algorithms (Shen et al., 2017a; Brignole et al., 2018). The most widely used supplementary measures include patient education, such as advice on avoiding triggers, recognition of prodromes and teaching manoeuvres that can abort an impending syncopal episode. In some patients, drug therapy can complement these measures, while cardiac pacing is reserved for specific groups (Shen et al., 2017a; Brignole et al., 2018). The most significant limitation to the success of the usual treatments is the lack of total understanding of the pathophysiology of this syndrome, which involves complex mechanisms, including central, psychogenic and peripheral abnormalities.

Within the present chapter, we intended to address the benefits of a tilt training programme, designed specifically for reflex syncope patients. To the best of our knowledge, this is the first study that combines the assessment of cardiocirculatory parameters, cardiovascular autonomic function, baroreceptor reflex behaviour and QoL in patients with refractory syncope, subjected to a combined tilt training programme. In general, our results indicate that tilt training was beneficial for most patients, by significantly reducing the number of syncopal episodes and improving patients' QoL. This benefit was achieved through a significant increase in the vasoconstrictor reserve of these patients after nine tilt training sessions and by modulating the autonomic nervous system and the baroreflex function. Indeed, our results show that tilt training significantly increases autonomic tone (both sympathetic and parasympathetic) and

improves the baroreflex function. Besides, we clearly defined two main profiles of patients in accordance to their response to tilt training, which is a valuable component of our data, so as to later develop prediction tools regarding the usefulness of a personalised tilt training programme for the treatment of reflex syncope.

The first authors reporting the beneficial effects of repeated tilting on the recurrence rate of vasovagal syncope speculated that this response could be related to a combination of factors, including natural history (with long symptom-free periods being frequent), reassurance and coaching on appropriate postural manoeuvres to prevent presyncope from progressing to syncope (Morillo, Leitch, Yee, & Klein, 1993). Tilt training was first described in 1998 (Ector et al., 1998), reporting, at that time, the absence of recurrence of syncope for more than half a year in vasovagal syncope patients treated with sessions of 30 minutes of tilting -up twice a day. It was, then, hypothesised that repeated exposure of the cardiovascular system to orthostatic stress would exert a therapeutic effect on the regulatory mechanisms of cardiovascular reflexes. Since these initial findings, tilt training has been considered as an option for the treatment of malignant and recurrent vasovagal syncope in some clinical settings (Ector et al., 1998; Reybrouck et al., 2000), with each author defining its specific protocol, regarding the number and session duration.

Nevertheless, later studies were unable to report a beneficial usage of tilt training in reflex syncope patients, which may be due to the quite different used criteria to define the “respondents group” (Shen et al., 2017a), or the type of the study (Ector et al., 2005; Gajek et al., 2006; On et al., 2007; Duygu et al., 2008; Verheyden, Ector, et al., 2008). In fact, a recent meta-analysis assessing the role of tilt-training in the treatment of 500 patients with vasovagal syncope distributed by 8 studies (Di Girolamo, Di Iorio, Leonzio, et al., 1999; Abe et al., 2002; Foglia-Manzillo et al., 2004; Gajek et al., 2006; Gardenghi et al., 2007; On et al., 2007; Duygu et al., 2008; Zeng et al., 2008), four of them randomised (Abe et al., 2002; Foglia-Manzillo et al., 2004; Gajek et al., 2006; Zeng et al., 2008), showed tilt training effectiveness in preventing vasovagal syncope recurrence (n=500, OR 0.30, CI 0.15–0.61, p <0.05) (Vyas et al., 2013). However, the significant effect was lost when only randomised studies were considered (n=298, OR 0.47, CI 0.21–

1.05, p=0.07). In accordance, both American College of Cardiology (Shen et al., 2017a) and European Society of Cardiology (ESC) guidelines on syncope (Brignole et al., 2018) advert for the limited use of this therapy, due to the failure of randomised trials in demonstrating the worth of tilt training. Furthermore, several studies have pointed out certain limitations to tilt training, the primary one being, patients' compliance with a long treatment period (Foglia-Manzillo et al., 2004; Gurevitz et al., 2007), which is relevant, particularly, for patients who become asymptomatic after a few sessions. In these cases, education and motivation are crucial additional measures.

Other issues are related to whether tilt training aims are to bring about changes that enable individuals to improve their daily lives or to prepare the individual to react to intense acute stimuli, such as the ones enacted by a head-up tilt test. As an example, while Foglia-Manzillo and co-workers (Foglia-Manzillo et al., 2004) used a positive tilt test one month after tilt training as the endpoint, other authors found results similar to ours (Reybrouck et al., 2000, 2002; Abe, Kohshi, & Nakashima, 2003b; Abe, Sumiyoshi, et al., 2003). Specifically, Abe and collaborators observed no spontaneous or orthostatic stress-induced syncopal episodes after tilt training, while Reybrouck and colleagues reported a significantly lower number of episodes after tilt training complemented by home self-training. They also reported that patients who did not complete the tilt training programme had no episodes of syncope up to a year after dropping out, which suggests that autonomic balance had already been restored in these patients.

Due to all these controversial results and conclusions, the present study attempted to develop a standardised protocol of tilt training. The significant differences include precise exclusion/inclusion criteria, the training protocol and the endpoints chosen, which may tend to lead to differences in results interpretation and, hence, in the evaluation of the efficacy of the tilt training.–Regarding the protocol differences, in opposition to other studies, we proposed a combined orthostatic training programme, composed of two branches: in-hospital tilt training sessions and daily self-training sessions at home.

In the present study, with an average follow-up of 37.7±11.2 months, all patients completed all the in-hospital sessions. Nevertheless, after a one-year follow-up, 24% of them had stopped home self-training. At the moment, with 47 (46%) patients having completed 48 months of follow-up, 39 patients (38%) have ceased home self-training due to symptomatic improvement. After the 1st tilt training programme, most of the patients did not present recurrence of syncope episodes (n=89; 86,3%) or pre-syncope (n=82, 80%) and reported a significant improvement of QoL. The overall long-term compliance of our study is better than the one reported by other authors. Two studies reported poor compliance (Gajek et al., 2006; On et al., 2007) due to low motivation of patients, in particular, those with less frequent symptoms, to continue training after the initial weeks.

Regarding motivation, in our study, patients were contacted once a month for the first six months and then yearly, to be recalled about the importance of maintaining constant training, even in the absence of symptoms. The frequency of tilt training sessions is another factor to take into consideration in the adherence of our patients to the training protocol. In our study, we recommended three weekly sessions, which certainly eased the maintenance of training in our patients and the clinical team control over the adequate performance of the sessions.

An important aspect to be discussed in handling vasovagal syncope is the beneficial effect of simple clinical follow-up on the recurrence of symptoms and patients' anxiety levels. In previous studies (Kapoor, 1992; Kapoor, Fortunato, Hanusa, & Schulberg, 1995; Ventura et al., 2001; Kouakam et al., 2002; Lee et al., 2013; Rafanelli, Gostoli, Roncuzzi, & Sassone, 2013) the presence of at least one psychiatric diagnosis (depression or panic syndrome), relates to a higher number of recurrences in patients with vasovagal syncope. Moreover, elevated levels of anxiety and impairment of QoL are observed in this group of patients, due to frequent recurrences, especially before diagnostic clarification (Linzer et al., 1991). In our study, a constant apprehension of having syncope recurrences had a significant impact on patients' QoL, with severe functional impairment previously to the starting of the tilt training programme. This explains why a vast majority of patients kept long-term compliance with the self-training part of the

protocol at home. We could observe that patients were encouraged by a regained self-confidence brought up during the in-hospital phase of the programme. After tilt training, patients obtained sustained benefits in QoL, as measured by the ISQL scale. In the tilt training responders' group, at 12-months follow-up, the tilt training programme was associated with a QoL improvement, in the ISQL items related to worry, fear and frustration with difficulties experienced. This improvement was probably linked, not only to the decrease in the number of syncopal episodes, which undoubtedly influenced positively in the anxiety levels of this population, but also to the introduction of a specific therapeutic measure and its physiological modifications. It should also be recalled that all patients involved in this study were already refractory to general measures before inclusion in the tilt training programme. The non-responders' group only achieved minor improvement in the QoL, which may indicate that the presence of at least one episode of pre-syncope impairs the patient's ability to live a healthy life.

Although the clinical efficacy of tilt training has been demonstrated, the physiological mechanisms underlying the improvement in these patients' condition are not entirely clear. Previous studies have indicated that humoral mechanisms (e.g., the renin-angiotensin system (Gajek et al., 2009), baroreflex changes, and overall autonomic alterations may be involved in the observed responses (Abe, Kohshi, & Nakashima, 2003a; Verheyden, Ector, et al., 2008; Gajek et al., 2009; Tan et al., 2010). In particular, Verheyden and co-workers (Verheyden, Ector, et al., 2008) observed an increased vasoconstrictor reserve, without changes in baroreflex or autonomic tone, while others showed an increase in HF and LF bands, which are indicators of increased autonomic tone (Tan et al., 2010).

In the present study, we relate hemodynamic changes to modifications in autonomic tone and qualitative and quantitative modulations of the arterial baroreceptor reflex. In our results, a significant increase in mean sBP, stroke volume and cardiac output, total peripheral resistance, and LF_{SBP} and HF_{RRI} were observed. This implies that the increase in total peripheral resistance is mainly due to increased sympathetic activity in the vascular system, which maintains BP without significant variations during postural changes. At the same time, and as expected, a reduced chronotropic response was

developed, echoed by an increased HF_{RRI}, BRS and BEI, translating an improved ability to adapt to changes in posture. Besides these absolute changes in total peripheral resistance, it is also worth noting the decrease in its standard deviation, which we take as a reflection of peripheral and somatic sympathetic remodelling, enabling the individual to regain homeostatic power to better adapt to postural changes, probably translating empowerment of patients' vasoconstrictive and sympathetic reserves. Furthermore, although not analysed in the present study, tilt training may also increase muscle tone in the lower limbs, which, together with the increase in total peripheral resistance, would improve postural adaptation.

In the previous chapter, we demonstrated an evident diminution of global baroreceptor responsiveness accompanying an evolving vasovagal syncope, characterised by both a qualitative (BRS) and quantitative (BEI) impairment of heart rate control, associated with an increased latency for the baroreceptor induced chronotropic response. In the current study, while in the supine position, no differences were found between the first and the ninth tilt training session, with patients having similar BEI values. This is in agreement with other authors, which reported an unchanged baroreflex control in the supine position in patients undergoing tilt training (Verheyden, Ector, et al., 2008). However, after head-up tilt, a significant increase in the BEI values was noted at the end of the tilt training programme. Also, an improved coherence and latency of the baroreflex arch could be recognised at the end of the tilt training programme.

In a subgroup analysis, patients with increased BEI value as a response to the tilt training programme achieved a positive response to the tilt training programme, translated by a reduction in symptoms and improvement in the quality of life. Non-responders presented statistically lower values of BEI at the 5^{th} minute of the 9^{th} tilt training session, and significantly smaller improvements of these values when compared to the same period of the first tilt training session. In our cohort, a BEI cut off value > 57.5 at the first five minutes of head-up tilt on the ninth tilt training session had an AUC of 0.9023 ($p< 0.0001$), with a sensitivity of 89.89% and specificity of 84.62% in predicting response to tilt training programme. This implies that a lower BEI value at the end of the tilt training programme is indicative of a higher possibility of the patient to be a non-responder to

tilt training and, hence, to have syncope recurrence. There is only another study found in the literature dealing with the prognostic role of the baroreflex in tilt training outcomes, which reveals that responders to tilt training had significantly higher BRS values in supine position when compared with non-responders, with a BRS value < 8,94 ms/mmHg being the only predictor of tilt training non-response in a multivariate analysis (Chun et al., 2016). In opposition to these data, we have not found significant BRS or BEI differences between responders and non-responders in the supine position but documented significant differences in the orthostatic position, which may be related to the proper role of the baroreflex, that is, a rapid physiological adaptation of blood pressure to postural changes.

In an in-depth analysis of the non-responders, an improvement trend during tilt training could, nevertheless, be seen: BEI and coherence were still improving at the end of the 9th tilt training session, when the training session was, by protocol, interrupted. This may allow speculating about the usefulness of autonomic and baroreflex information to preselect patients who are most likely to benefit from tilt training and to precisely define the duration of the tilt training, in a patient-tailored training.

The main limitation of the study is the absence of a proper control group or placebo training group. Results could be influenced by the spontaneous reduction in the frequency of syncopal episodes after evaluation. However, the sequential comparison of haemodynamic and autonomic parameters showed clear significant improvements. In the QoL assessment subgroup, two control groups were set up (a 'general measures plus counterpressure manoeuvres' and 'no structured approach' group); patients' characteristics on the three groups were similar. Tilt training responders showed not only symptomatic benefits but also the improvement of the quality of life, while in the general measures plus counterpressure manoeuvres group, despite a non-significant reduction of the number of syncopal episodes, most patients were symptomatic and without a significant improvement in QoL. It should also be recalled that all tilt training patients involved in this study were severely symptomatic and were already refractory to general measures before inclusion in the tilt training programme.

In conclusion, in patients with refractory vasovagal syncope, our training protocol demonstrated to be an effective therapeutic option, with long-term benefits regarding the higher tolerance to orthostatism and improved QoL, through three main mechanisms: an increase with lower variability of the vasoconstrictor reserve, an overall rise in autonomic tone and beneficial changes in baroreflex sensitivity.

ORTHOSTATIC CHALLENGE IN PATIENTS WITH RECURRENT SYNCOPE AND OTHER CARDIOVASCULAR CO-MORBIDITIES

During the enrolment process of the previous work, we came across with patients with recurrent syncope but who did not meet the inclusion criteria. Here, we describe an emblematic case of a patient with recurrent syncope and symptomatic episodes of atrial fibrillation, in whom a tilt-training programme was effective in abolishing not only the syncopal episodes but also atrial fibrillation while increasing the coherence between blood pressure and heart rate.

Case report

A 37-year-old man, surfer, suffering from recurrent syncope during the last 5 years, most of the times induced by ingestion of cold drinks. He was referred to a cardiology unit due to an episode of syncope followed by irregular palpitations. The ECG showed atrial fibrillation (AF) with a ventricular rate of ±150 bpm that was converted to sinus rhythm after i.v. flecainide. Echocardiogram evaluation was considered normal. During exercise stress testing, with an 18-minutes duration of Bruce protocol, he attained 93% of the maximum expected heart rate for the age, and atrial fibrillation was not documented. Holter recording (24 h) evidenced sinus arrhythmia, with a heart rate ranging between 35 and 103 bpm (mean 57 bpm), with two sinus pauses (2.0 seconds duration) during sleep. Heart rate was <50 bpm and <40 bpm in 44% and 14% of the recording, respectively, without documentation of atrial fibrillation. The patient also took an event recorder that showed sinus rhythm, with a heart rate ranging between 33 and 81 bpm during manual activation due to nonspecific “sensations”. Finally, the head-up tilt testing induced a cardioinhibitory reflex syncope after sublingual nitroglycerin, with a sinus arrest of 18 seconds, without atrial fibrillation.

Upon discussion of the treatment options, the patient refused to undergo antiarrhythmic drug therapy or atrial fibrillation catheter ablation. He had a CHADSVASC score of 0 and, therefore, aspirin (150 mg/day) was prescribed. However, the patient accepted to be included in a tilt-training programme as previously described (Laranjo et

al., 2012). In brief, this programme encompasses nine head-up tilt sessions (3×/week for 30 minutes; 6 sessions tilted at 60° and the remaining ones at 70°), with continuous blood pressure and ECG monitoring at the hospital lab. These orthostatic stress-induced manoeuvres were complemented with self-induced orthostatic manoeuvres at home (standing every day against a wall at 20°, for 20 min) and with a lifting of the bed head at 10°. Orthostatic self-induced manoeuvres at home were maintained regularly for 1 year. During a five years follow-up period, the patient did not show recurrence of syncope or clinical atrial fibrillation, despite no other therapy. All the procedures were executed in accordance with the Declaration of Helsinki, and informed consent was obtained from the patient.

Autonomic signal analysis was performed by applying, simultaneously, the wavelet coherence technique to both HR and SBP (Keissar et al., 2006; Keissar, Davrath, & Akselrod, 2009; Keissar et al., 2010). The dynamic trend of the wavelet coherence technique was demonstrated for the last 2 min of the supine condition and for the first 5 min of head-up tilting. In baseline conditions, before the first tilt-training session (Figure 39-A), the patient showed a reduced global coherence between HR and sBP variations (wavelet coherence score of 0.28). During the tilt-training programme, a gradual but statistically significant increase of wavelet coherence values was observed (Figure 39B–D). At the end of the ninth tilt-training session, the patient presented a supine wavelet coherence score of 0.62, with an orthostatic score of 0.59 (Figure 40). Hemodynamic and autonomic parameters were obtained from continuous monitoring (Task Force System, CNSystems, Austria).

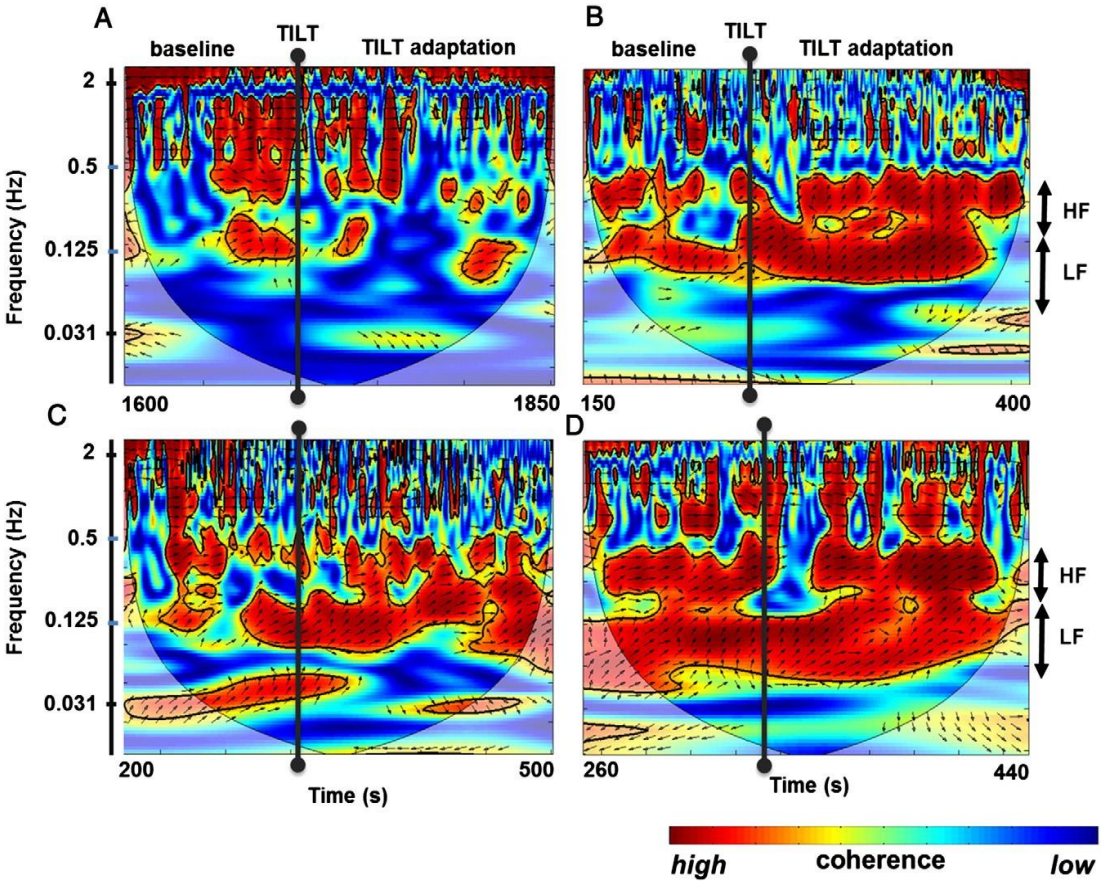


Figure 39. Modification of HR and SBP variability coherence along the training period
(A: basal conditions before training program; B, C and D: 1st, 4th and 9th tilt-training sessions, respectively). The increase of coherence during the sessions, which relates with an increase of baroreceptor remodelling, is represented by an improvement of the band organization together with a higher density of the orange/red.

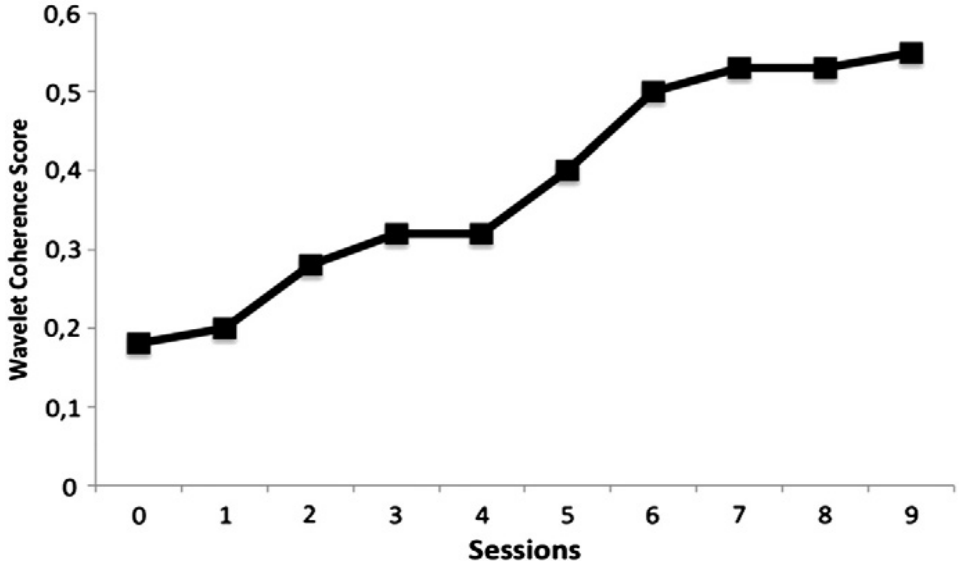


Figure 40. Serial values of the wavelet coherence score for the first 5 min of head-up tilt over the nine tilt-training sessions.

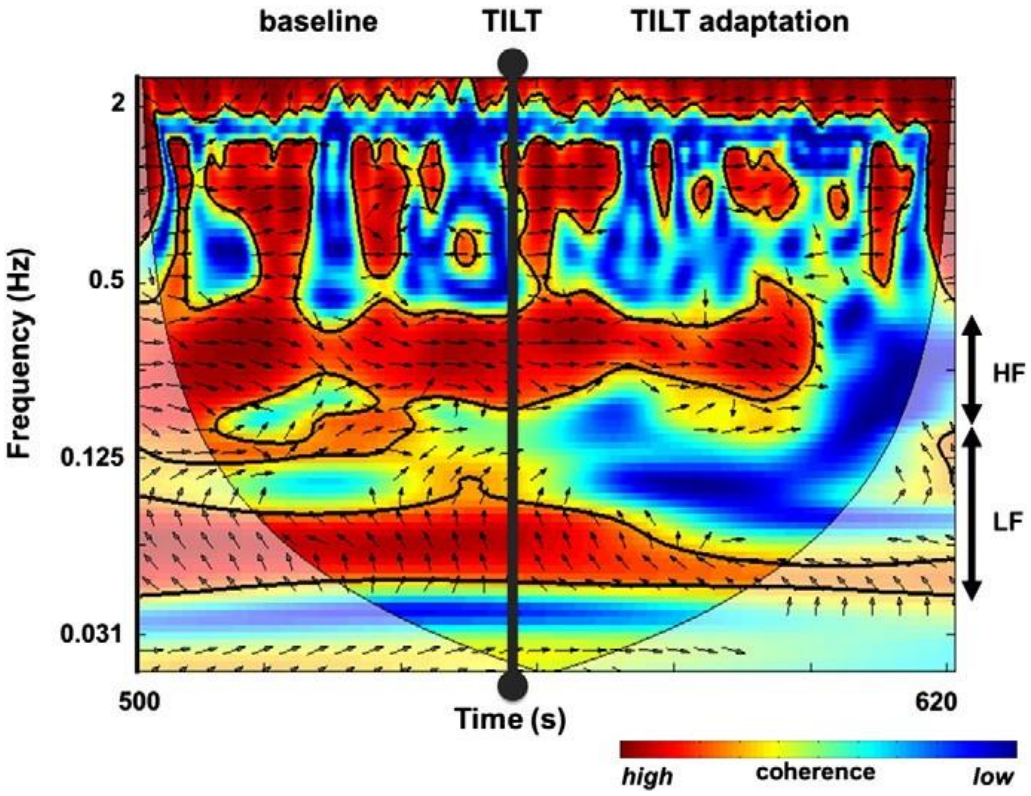


Figure 41. In this picture are depicted the changes in coherence evoked by a tilt manoeuvre in a healthy subject. *After tilting (vertical black line), there is a drop of coherence, which reaches its minimum value approximately 20 s after tilting, recovering later to a significantly lower value*.

To our knowledge, this is the first report of a patient suffering from recurrent reflex syncope and paroxysmal atrial fibrillation who shows a long-term clinical improvement after a program of tilt-training, a non-invasive autonomic modulatory scheme. The present work also confirms that wavelet coherence analysis is a feasible technique to study integrated changes in autonomic cardiovascular balance induced by head-up tilting.

The co-existence of paroxysmal atrial fibrillation and reflex syncope in the same patient has been scarcely reported (Brignole et al., 1993). The autonomic nervous system, particularly its parasympathetic branch, has been implicated in the abnormal reflex response, namely the one evoked by the ingestion of cold drinks, and could be related with pathophysiological mechanisms underlying these clinical situations.

In fact, vagal stimulation significantly affects the electrophysiological properties associated with susceptibility to atrial fibrillation, such as conduction velocity, action potential duration and atrial refractoriness, creating the conditions for the occurrence of atrial arrhythmias (Oliveira et al., 2011). Sudden fluctuations in autonomic activity can be a determining factor in paroxysmal atrial fibrillation. In fact, several authors described significant alterations in sympathetic and/or vagal tone prior to paroxysmal atrial fibrillation episodes (Ogawa et al., 2009; Arora, 2012; Carnagarin, Kiuchi, Ho, Matthews, & Schlaich, 2019). A clinical pattern of vagally mediated paroxysmal atrial fibrillation has been observed, mainly in young adults without associated heart disease.

Similar sudden fluctuations of autonomic nervous outflow have also been demonstrated during orthostatic stress, in patients with reflex syncope, as demonstrated in *Chapter 5*. Accordingly, we have previously shown, in this Chapter that, in these patients, tilt-training may be an effective therapeutic option, with long-term benefits.

In the present report, after nine tilt-training sessions, an increase in vasoconstrictor reserve with a significant increase in baroreflex sensitivity determined by wavelet coherence technique has been observed. These changes imply an increase, over time, of sympathetic outflow together with an improvement of parasympathetic function. Moreover, although not evaluated in this patient, we can speculate that tilt-training could also induce an increased muscle tone of the lower limbs, which would complement the increase in total peripheral resistance, allowing a better postural adaptation. Through the application of the wavelet coherence technique, it was possible to follow and document the trend of changes in HR and sBP variability evoked by the consecutive orthostatic challenges, which mainly activate and modify the baroreflex activity and, thus, lead to modifications on both HR and sBP and their relative interactions. The relationship between these two variables, as is clearly seen in the figures, approaches along time those observed in healthy subjects, which indicates, in our opinion, modulation of the autonomic activity to the periphery, particularly to the cardiovascular system, through the baroreflex. This phenomenon lies in accordance with the clinical outcome of this patient that has not shown recurrence of syncopal or paroxysmal atrial

fibrillation episodes until the present, i.e., during the 5-year period of follow-up. In conclusion, we strongly suggest the application of a tilt-training programme adjusted to the patient's history, as a therapeutic option in similar clinical conditions, as a way of modulating the autonomic function in a non-invasively way.

CHAPTER 7

CHAPTER 7

CONCLUSION

I. Summary of main results

At the end of this translational work, our major findings are:

1. On the syncope mechanisms:
    a. We showed that a functional asynchrony of hemodynamic and autonomic reflex responses are mechanisms underlying syncope pathophysiology; this is conveyed by a temporal mismatch between cardiac output and total peripheral resistance adaptations, together with a delayed baroreflex response, accompanied by an unbalanced increase of autonomic tone;
    b. Regardless the VASIS type of syncope, cardiac output, total peripheral resistance and autonomic nervous system activity, via baroreceptor function and changes in sympathetic and parasympathetic tone concur without success to maintain consciousness in all fainters; this failure might be due to a transient autonomic impairment, which is expressed by a decreased sympathetic reserve;
    c. Based on our protocol and novel data analysis, we defined a predictive algorithm, which by taking into account the evolution of cardiovascular and autonomic parameters during the supine and first five minutes of head-up tilt, is able to predict the head-up tilt test outcome, obviating the need for the patient to develop syncope, thus avoiding the stress of this event and improving value-based healthcare, due to the improvement of results and decreased consumption of resources and laboratory time usage.

2. On the orthostatic training:

a. We showed the effectiveness of a non-invasive, non-disruptive and integrative neuromodulation strategy of the autonomic and cardiovascular variables involved in the syncopal mechanism, leading to a substantial increase of patients' quality of life, with the abolishment or a significant decrease of the number of syncopal events in the vast majority of the enrolled patients;

b. We designed and implemented an orthostatic training program to be performed at home and at the hospital, with patient compliance and an in-depth integrative and dynamic evaluation of haemodynamic and autonomic parameters in order to achieve personalization. This orthostatic training showed to be an effective therapeutic option for recurrent syncope patients;

c. We demonstrated the potential of a tilt training programme to be applied to other diseases running with syncope or other manifestations of cardiovascular autonomic dysfunction.

3. On the new dynamic tools for autonomic evaluation:

   a. A new software – FisioSinal – integrating innovative methods for autonomic evaluation was developed and implemented; time-scale and phase methodologies offer new insights into the dynamic evaluation of autonomic function in reflex syncope patients, helping to timely risk-stratify these patients and enabling timely appropriate therapeutic measures to be pursued;

   b. A user-friendly computing platform, including both hardware architecture and software framework, was designed, tested and implemented for autonomic evaluation. This platform allows to import and process data in a multivariate, integrated and standardised way; data visualisation tools use a standardised methodology to follow the best practices, precise classification of visualisations and data profiling, based on the needs of the clinician or the human physiologist. Among the various features are the ability to choose among several graphical dashboards, the accuracy of trend tracking

capability, related to dynamic data analysis, a high level of security, following general data protection regulation (GDPR) and a simplified software interface.

II. Discussion of the hypotheses under study

The transient reduction in global cerebral perfusion pressure is behind the transient loss of consciousness, which is the hallmark of syncope. Syncope is a common manifestation in clinical practice, with a lifetime prevalence of 30% to 40% in the general population, and with a tremendous negative impact on patients' quality of life. History taking, ECG and tilt test are critical tools to differentiate syncope from other causes of TLOC. The fact that syncope might be a manifestation of an underlying deadly condition, together with the lack of consistent terminology for syncope and related disorders, accounts for a defensive medical evaluation, which makes admissions for syncope common with the consequent increase in related health costs.

Regarding syncope's pathophysiology, there are several factors which have been implicated in the development of the syncopal episode, but its mechanisms of initiation, maintenance and termination are not yet fully understood. One of the contributors to these mechanisms is the autonomic nervous system, which, together with the endocrine system, is responsible for the maintenance of the body homeostasis and the individual´s survival. Several authors have reported that impairment of autonomic function is observed in syncope patients, together with modifications of the baroreceptor reflex, an autonomic reflex that controls arterial blood pressure in each instant, as well as the total peripheral resistance. However, the way how the autonomic nervous system behaves during syncope and its associated-autonomic reflexes are not consensual.

Concerning reflex syncope, despite the usefulness of the available pharmacological and non-pharmacological approaches, the therapeutic options available are few, vexing and

mainly palliative rather than curative. Thus, the medical challenge is to provide the best, most accurate therapeutics, tailored to each individual patient. Patients' education and their reassurance about the benign nature of the syncopal events are necessary actions to secure their compliance with the prescribed treatment.

In the present work, we addressed the relation of the autonomic nervous system with reflex syncope: firstly, by showing the autonomic influence in the pathophysiological mechanisms of reflex syncope and, secondly, by demonstrating how the non-invasive modulation of this system could positively impact the quality of life of syncope patients through the abolishment or substantial decrease of syncopal events.

For that, we developed the FisioSinal framework, where dynamic methodologies of autonomic evaluation through signal processing were implemented. Indeed, the usage of wavelets or Hilbert Huang transform allows for the quantification of autonomic function by the assessment of heart rate and blood pressure variability along time, bypassing the need of a stable signal and 5 minutes-long recordings, requisites of the classical Fourier analysis. This framework also supports the data analysis of the full battery of autonomic manoeuvres. We tested and validated our results against the gold standard methodologies of autonomic evaluation in healthy subjects and patients, of both genders and various age groups. The result of this development is the FisioSinal platform, a novel modular approach that provides a tailored, personalised, analysis instrument for the time- and cost-effective autonomic evaluation.

FisioSinal also produces single-case summary reports of autonomic function, including graphical maps under a secure, data collection and analysis platform, compliant with GDPR. FisioSinal is very dynamic in both its usage and nature thus, beyond the present work, other features have been added (e.g., stochastic analysis, auto-regression, baroreflex analysis, application to animal data), now being available for clinical and physiological studies of various natures, facilitating personalised medicine, interprofessional collaboration and multicentre research.

Under this scope, the FisioSinal platform was crucial for pursuing our goal to dive into the autonomic role in reflex syncope mechanisms. Thus, in paediatric and adult patients with recurrent reflex syncope, we investigated the profile of the autonomic and cardiocirculatory responses evoked by head-up tilt. In both groups, results were similar, but the paediatric patients showed a larger amplitude of changes between the baseline and the provoked response, which agrees with the physiological modifications related to ageing processes. Nevertheless, the subjects of both populations fell into two groups upon their tilting behaviour: those who fainted and those who did not. In the fainters' groups, along with the various phases of the tilt response, we were able to describe instantaneous variations of cardiac output, which almost reached a 50% decrease over the 4 phases of syncope, associated to peripheral total resistance modifications. These results, despite being in accordance with previous works, were observed in different time points of the syncopal event and together with the results from autonomic and baroreflex evaluation confirmed that the mechanism behind this physiological inability is nurtured by an autonomic impairment defined by a prolonged time delay to the baroreflex response.

Subjects who best tolerated the induced orthostatic stress showed a shorter baroreflex latency, which is a characteristic of healthy individuals, implying that a fast baroreflex response is a crucial factor in response to gravitational stress, and a delayed baroreflex response is a contributor to the loss of the functional synchrony needed to keep consciousness. Thus, we may conclude under the integration of all our data that reflex syncope might result from a primary transient autonomic impairment, whose core manifestation is an impairment of the arterial baroreceptor reflex, which is not able to compensate the drop in blood pressure due to the absence of sympathetic reserve. The origin of this dysfunction is still a conjecture since reflex syncope spans from young ages until aged adults. We may speculate about a transient impairment of the autonomic nervous system in patients with recurrent syncopal episodes. In these cases, the system (over)reacts upon the presence of specific triggers. Therefore, a system modulation to reach a point of less variation is advisable.

How to effectively modulate a system that expresses itself along the various body functions is still an unresolved issue. Invasive cardiac neuromodulation is a technique that involves radiofrequency ablation of ganglionic plexuses located on the epicardial surface of the heart (Pachon et al., 2005, 2011; Yan et al., 2012; Aksu, Golcuk, Yalin, Guler, & Erden, 2016; W. Sun et al., 2016). In non-randomized studies, with an exceedingly small number of symptomatic patients, a benefit was suggested in controlling the recurrence of syncopal episodes. More data on the physiological mechanisms underlying the cardiac intrinsic nervous system, the exact relation between syncope and the underlying cardiac rhythm and properly defined outcomes are needed before the massification of this procedure with yet unpredictable results.

Bridging with the previous concept, one of the challenges in recurrent syncope management is to reach therapy effectiveness. Due to the intermittent and variable, subject to subject, nature of syncope, no therapy has been proven consistently effective in preventing recurrences. Patients' education, application of counterpressure measures, volume support, and beta-blockers and other medications are among the available therapeutic measures. Some patients with recurrent syncope respond poorly to general measures. Orthostatic training may be an effective approach, although study results have been conflicting due to the variations in the established protocol and data analysis.

In our study, by performing a novel integrated evaluation of the patients, we can conclude that the tilt training protocol used is an effective therapeutic option, with long term benefits for patient quality of life. Besides, tilt training may also be of utility when other cardiovascular co-morbidities also run with recurrent syncope, or in other cardiovascular disorders in which the autonomic nervous system may play a pivotal role, such as some forms of cardiac arrhythmias. This is a novel approach that takes advantage of the plasticity of the neural tissue to shape it to our advantage, without injuring it. It also allows to speculate about the common central origin of various diseases that ultimately may be treated by tackling specific areas of the central

autonomic network in a direct, and probably more effective way or just by peripherally modulating the autonomic nervous system reflex arc.

Despite the need for further studies targeting this concept, we can express that in patients with refractory vasovagal syncope, our training protocol, which induces a positive autonomic modulation, is an effective and personalised therapeutic option, with long-term benefits to patients and their quality of life.

In conclusion, the primary difficulty in dealing with reflex syncope and its recurrences lies in the inherent nature of this condition. With our work, we approached the investigation of reflex syncope pathophysiological mechanisms and the orthostatic training as a complementary therapeutic measure, and also how they are imprinted by autonomic function. Nevertheless, there is much left to be done in order to understand this disease and to manage these patients in a personalised and multidisciplinary approach.

APPENDIX 1

SUPPLEMENTAR MATERIAL

FIGURES AND TABLES

CHAPTER 5

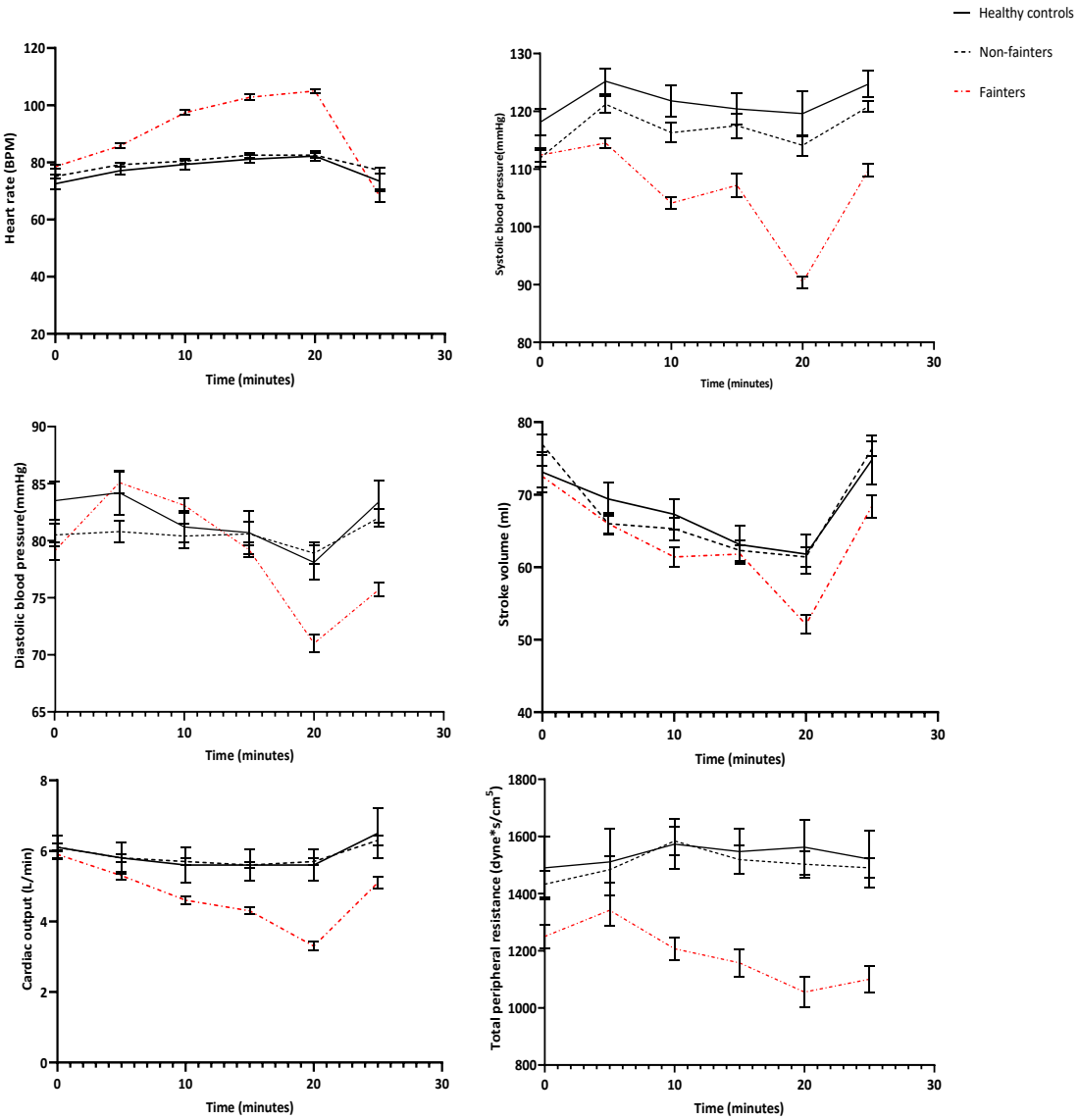


Figure 36. Comparison of the evolution of haemodynamic parameters along time during HUT in the healthy controls, non-fainters, and fainters groups.

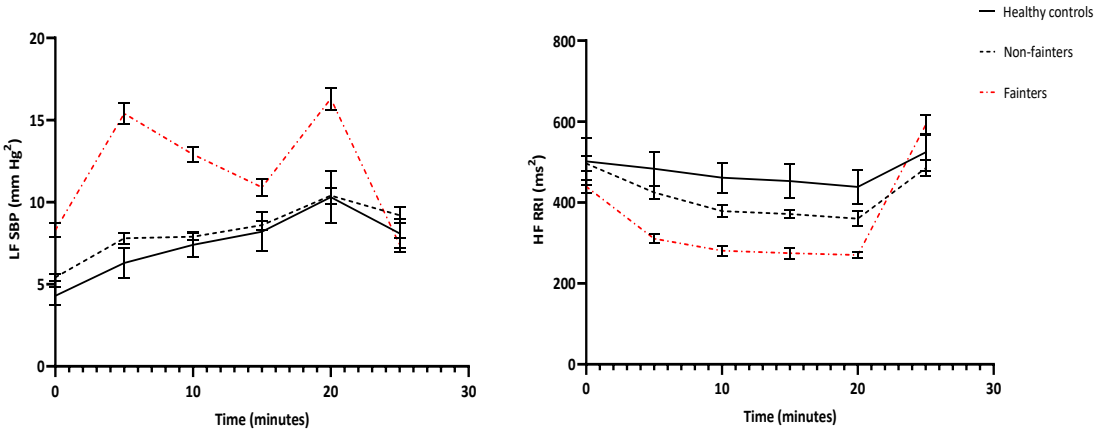


Figure 37. Comparison of the evolution of heart rate and blood pressure parameters along time during HUT in the healthy controls, non-fainters, and fainters groups. *LF – low frequency band; HF – High frequency band.*

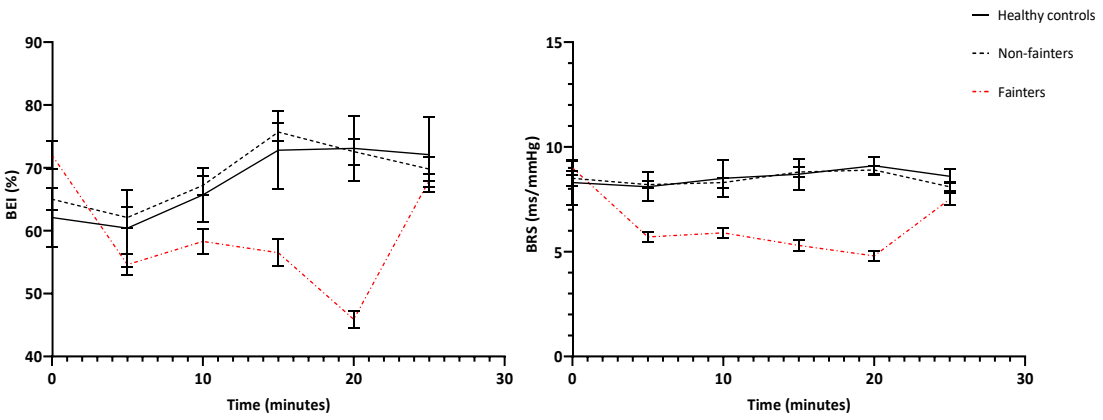


Figure 38. Comparison of the evolution of BEI and BRS parameters along time during HUT in the healthy controls, non-fainters, and fainters groups. *BEI – baroreflex effectiveness index; BRS – baroreflex sensitivity.*

Table 14. Comparison of the evolution of haemodynamic and autonomic parameters along time during HUT between the paediatric and adult fainters (first 10 minutes)

	Basal			0-5 min ('phase 1')			5-10 min ('phase 2')		
	Paediatric fainters (n=99)	Adult Fainters (n=188)	P	Paediatric fainters	Adult Fainters	P	Paediatric fainters	Adult Fainters	P
HR (bpm)	85.1±12.3	78.5±10	**0.0001**	96.4±23.5	85.8±11.7	**0.0001**	105.8±18.4	97.4±11.5	**0.0001**
SBP (mmHg)	96.2±8.4	112.4±16.1	**0.0001**	93.1±9.1	114.5±11.9	**0.0001**	98.8±11.4	104.1±13,1	**0.0008**
DBP (mmHg)	74.7±5.8	79.1±10.3	**0.0001**	79.3±10.4	85.1±12.5	**0.0001**	80.2±9.6	83.1±8.8	**0.0106**
SV (ml)	70.8±12.4	72.5±19.9	0.4395	63.7±14.6	61±18.	0.1995	60.1±19.5	61.4±18.2	0.5752
CO (l/min)	5.8 ±1.2	5.9±1.5	0.5667	5.1±2.7	5.3±1.5	0.4203	4.4±3.1	4.6±1.5	0.4620
TPR (dyne*s/cm^5)	1131.2±452.6	1250.1±576.3	0.0756	1274.1±617.8	1341.5±729.4	0.4342	1200.4±502.7	1206.7±562.8	0.9256
LF_{SBP} (mm Hg2)	6.8±6.1	8,3±5.7	**0.0395**	19.4±10.2	15,4±8.5	**0.0005**	15.8±8.4	12,9±6,4	**0.0012**
HF_{RRI} (ms2)	932,8±620,4	439,2±208,2	**0.0001**	517.4±305.1	310,4±164,5	**0.0001**	427.1±281.3	280,7±171,4	**0.0001**
BEI (%)	79±35.2	72±30.2	0.0793	65.2±28.5	54.6±23.1	**0.0008**	63.8±22.7	58.3±26.8	0.0831
BRS (ms/mmHg)	10.2±6.5	9±4.7	0.0739	7.1±4.3	5.7±3	**0.0014**	6.4±5.1	5.9±3.4	0.3228

HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; LFSBP: Low-frequency band derived from SBP; HFRRI: High-frequency band power derived from R-R intervals; significance for p<0.05

Table 15. Comparison of the evolution of haemodynamic and autonomic parameters along time during HUT between the paediatric and adult fainters (last 10 min and Tilt-down)

	10-15 min ('phase 3')			15-20 min ('phase 4')			Tilt-down		
	Paediatric fainters	Adult Fainters	P	Paediatric fainters	Adult Fainters	P	Paediatric fainters	Adult Fainters	P
HR (bpm)	114.9±25.2	102.8±13.2	**0.0001**	126.4±.30.2	105±10.3	**0.0001**	80.8±16.3	68±25.1	**0.0001**
SBP (mmHg)	100.3±10.5	107.2±28.7	**0.0216**	88.7±13.9	90,4±14.7	0.3436	98.1±9.5	109,8±14.7	**0.0001**
DBP (mmHg)	77.4±8.1	79.2±9.2	0.1021	70.4±7.7	71±10.3	0.6109	78.5±9.1	75.7±8.4	**0.0096**
SV (ml)	58.7±15.8	61.8±17.3	0.1384	55.2±18.1	52.1±17.8	0.1643	63.7±13.4	68.4±21.6	**0.0494**
CO (l/min)	3.9±2.5	4.3±1.3	0.0754	3.5±2.1	3.3±1.8	0.3994	5.4±1.7	5.1±2.3	0.2539
TPR (dyne*s/cm^5)	1127.2±628.4	1157.6±662.5	0.7071	1023.1±691.3	1055.4±711.7	0.7123	1175.8±614.2	1100.2±646.8	0.3391
LF_{SBP} (mm Hg2)	14.1±8.3	10,9±7,30	**0.0009**	16.9±11.7	16,3±9.2	0.6337	10.9±6.1	7,4±5.9	0.0001
HF_{RRI} (ms2)	389.7±206.4	274.7±181,6	**0.0001**	259.1±217.4	270,2±116,6	0.5736	572.2±295.1	591,8±348,1	0.6337
BEI (%)	55.9±21.3	56.5±29.2	0.8568	51.2±18.5	45.9±18.3	**0.0209**	72.8±20.3	68±14.7	**0.0224**
BRS (ms/mmHg)	5.8±4.8	5.3±3.6	0.3213	5.2 ±2.9	4.8 ±3.4	0.3205	9.3±6.8	7.5±4.1	**0.0056**

HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; LFSBP: Low frequency band derived from SBP; HFRRI: High frequency band power derived from R-R intervals; significance for $p<0.05$

Table 16. Comparison of the baroreflex sensitivity (sequence method) along time during HUT in the fainters group.

	Basal	0-5 min ('phase 1')		5-10 min ('phase 2')		10-15 min ('phase 3')		15-20 min ('phase 4')		Tilt-down	
	Value	Value	p	Value	p	Value	p	Value	p	Value	p
BEI (%)	72±30.2	54.6±23.1	0.0001	58.3±26.8	0.1524	56.5±29.2	0.5339	45.9±18.3	0.0001	68±14.7	0.0001
BRS (ms/mmHg)	9±4.7	5.7±3	0.0001	5.9±3.4	0.5457	5.3±3.6	0.0975	4.8 ±3.4	0.1670	7.5±4.1	0.0001

Table 17. Comparison of the evolution of haemodynamic and autonomic parameters along time during HUT between the healthy controls and non-fainters

	Basal			0-5 min ('phase 1')			5-10 min ('phase 2')		
	Controls (n=30)	Non-fainters (n=219)	P	Controls	Non-fainters	P	Controls	Non-fainters	P
HR (bpm)	72.5±10	75.1±9.7	0.1714	77.1±8.5	79.2±10.5	0.0105	79.3±9.2	80.4±12.7	0.6475
SBP (mmHg)	118.1±12.4	111.9±21.4	0.1225	125.2±11.9	121.2±21.6	0.3218	121.8±14,7	116.3±25.5	0.2496
DBP (mmHg)	83.5±9.1	80.5±15.3	0.2958	84.2±10.6	80.8±14.3	0.2107	81.2±7.5	80.4±16.4	0.7927
SV (ml)	73.1±15.2	76.9±20.9	0.3375	69.4±12.6	66±21.2	0.3923	67.3±11.7	65.3±23.3	0.6447
CO (l/min)	6.1±1.8	6.1±1.5	1	5.8±2.4	5.8±1.6	1	5.6±2.8	5.7±1.4	0.7526
TPR (dyne*s/cm^5)	1490.1±601.7	1432.7±702	0.67	1510.6±634.8	1483.7±696.5	0.8413	1573.1±481.6	1583.9±723.4	0.9368
LF_{SBP} (mm Hg2)	4,3±3.9	5,42±3.2	0.0816	6,3±5.1	7,8±4.9	0.1189	7,4±4,1	7,9±3,5	0.4733
HF_{RRI} (ms2)	501,6±317.7	496,5±275,1	**0.0001***	483,4±231,1	424,8±238,3	0.2140	461.4±205,9	378,5±221.7	0.0540

	10-15 min ('phase 3')			15-20 min ('phase 4')			Tilt-down		
	Controls	Non-fainters	P	Controls	Non-fainters	P	Controls	Non-fainters	P
HR (bpm)	81.1±7.3	82.5±12.4	0.5467	82.2±9.4	82.5±12.6	0.9001	73.4±15.2	77.2±15.8	0.2158
SBP (mmHg)	120.4±15.2	117.5±31.6	0.6216	119.6±21.2	114.1±26.5	0.2770	124,7±12.5	120.8±13.7	0.1410
DBP (mmHg)	80.7±10.3	80.6±15.4	0.9725	78.1±8.1	78.9±13.6	0.7536	83.4±10.2	82±11.9	0.5398
SV (ml)	63.1±14.6	62.3±20.7	0.8380	61.8±15.1	61.4±19.6	0.9145	74.8±18.5	76.3±15.3	0.6242
CO (l/min)	5.6±2.4	5.6±1.4	1	5.6±2.5	5.7±1.3	0.7309	6.5±3.9	6.3±1.9	0.8180
TPR (dyne*s/cm^5)	1547.1±428.2	1518.7±756.6	0.8409	1562.4±524.7	1502.7±684.8	0.6466	1520.8±537.1	1490.2±507.1	0.7585
LF_{SBP} (mm Hg2)	8,2±6,5	8,6±4	0.6385	10,3±8.7	10,4±7,25	0.9450	8,1±4.8	9,2±7,4	0.4297
HF_{RRI} (ms2)	453.1±237.3	371.6 ±150,2	**0.0107***	438.3±234.9	360,2±273,8	0.1379	524,1±248,2	484,8±305,6	0.5008

Table 18. Comparison of the evolution of haemodynamic and autonomic parameters along time during HUT between the fainters and non-fainters

	Basal			0-5 min ('phase 1')			5-10 min ('phase 2')		
	Fainters (n=188)	**Non-fainters (n=219)**	**P**	**Fainters**	**Non-fainters**	**P**	**Fainters**	**Non-fainters**	**P**
HR (bpm)	78.5±10	75.1±9.7	**0.0006**	85.8±11.7	79.2±10.5	**0.0001**	97.4±11.5	80.4±12.7	**0.0001**
SBP (mmHg)	112.4±16.1	111.9±21.4	0.7928	114.5±11.9	121.2±21.6	**0.0002**	104.1±13,1	116.3±25.5	**0.0001**
DBP (mmHg)	79.1±10.3	80.5±15.3	0.2878	85.1±12.5	80.8±14.3	**0.015**	83.1±8.8	80.4±16.4	**0.0439**
SV (ml)	72.5±19.9	76.9±20.9	**0.031**	61±18.4	66±21.2	**0.019**	61.4±18.2	65.3±23.3	0.0637
CO (l/min)	5.9±1.5	6.1±1.5	**0.1807**	5.3±1.5	5.8±1.6	**0.0001**	4.6±1.5	5.7±1.4	**0.0001**
TPR (dyne*s/cm^5)	1250.1±576.3	1432.7±702	**0.0048**	1341.5±729.4	1483.7±696.5	**0.0452**	1206.7±562.8	1583.9±723.4	**0.0001**
LF_{SBP} (mm Hg2)	8,3±5.7	5,42±3.2	**0.0001**	15,4±8.5	7,8±4.9	**0.0001**	12,9±6,4	7,9±3,5	**0.0001**
HF_{RRI} (ms2)	439,2±208,2	496,5±275,1	**0.0199**	310,4±164,5	424,8±238,3	**0.0001**	280,7±171,4	378,5±221.7	**0.0001**

	10-15 min ('phase 3')			15-20 min ('phase 4')		
	Fainters	Non-fainters	P	Fainters	Non-fainters	P
HR (bpm)	102.8±13.2	82.5±12.4	**0.0001**	105±10.3	82.5±12.6	**0.0001**
SBP (mmHg)	107.2±28.7	117.5±31.6	**0.0007**	90,4±14.7	114.1±26.5	**0.0001**
DBP (mmHg)	79.2±9.2	80.6±15.4	0.2762	71±10.3	78.9±13.6	**0.0001**
SV (ml)	61.8±17.3	62.3±20.7	0.7936	52.1±17.8	61.4±19.6	**0.0001**
CO (l/min)	4.3±1.3	5.6±1.4	**0.0001**	3.3±1.8	5.7±1.3	**0.0001**
TPR (dyne*s/cm^5)	1157.6±662.5	1518.7±756.6	**0.0001**	1055.4±711.7	1502.7±684.8	**0.0001**
LF_{SBP} (mm Hg2)	10,9±7,3	8,6±4	**0.0001**	16,3±9.2	10,4±7,25	**0.0001**
HF_{RRI} (ms2)	274.7±181,6	371.6 ±150,2	**0.0001**	270,2±116,6	360,2±273,8	0.0001

HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; LFSBP: Low frequency band derived from SBP; HFRRI: High frequency band power derived from R-R intervals; significance for $p<0.05$

Table 19. Evolution of the haemodynamic and autonomic parameters along time during HUT in the fainters group.

	Basal	0-5 min ('phase 1')		5-10 min ('phase 2')		10-15 min ('phase 3')		15-20 min ('phase 4')		Tilt-down	
	Value	Value	p	Value	P	Value	P	Value	P	Value	p
HR (bpm)	78.5±10	85.8±11.7	**0.0001**	97.4±11.5	**0.0001***	102.8±13.2	**0.0001***	105±10.3	0.0724	68±25.1	**0.0001***
SBP (mmHg)	112.4±16.1	114.5±11.9	0.1512	104.1±13,1	**0.0001***	107.2±28.7	0.178	90,4±14.7	**0.0001***	109,8±14.7	**0.0001***
DBP (mmHg)	79.1±10.3	85.1±12.5	**0.0001**	83.1±8.8	0.0736	79.2±9.2	**0.0001***	71±10.3	0.0001	75.7±8.4	**0.0001***
SV (ml)	72.5±19.9	61±18.	**0.0011**	61.4±18.2	0.9313	61.8±17.3	0.8272	52.1±17.8	**0.0001***	68.4±21.6	**0.0001***
CO (l/min)	5.9±1.5	5.3±1.5	**0.0001**	4.6±1.5	**0.0001***	4.3±1.3	**0.0389***	3.3±1.8	**0.0001***	5.1±2.3	**0.0001***
TPR (dyne*s/cm^5)	1250.1±576.3	1341.5±729.4	0.1784	1206.7±562.8	**0.0456**	1157.6±662.5	0.4301	1055.4±711.7	**0.0156**	1100.2±646.8	0.5234
LF_{SBP} (mm Hg2)	8,3±5.7	15,4±8.5	**0.0001**	12,9±6,4	**0.0014***	10,9±7,30	**0.005***	16,3±9.2	**0.0001***	7,4±5.9	**0.0001***
HF_{RRI} (ms2)	**439,2±208,2**	**310,4±164,5**	**0.0001**	**280,7±171,4**	**0.0873**	**274.7±181,6**	**0.7420**	**270,2±116,6**	**0.7751**	**591,8±348,1**	**0.0001***

HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; $LF_{SBP:}$ Low frequency band derived from SBP; HF_{RRI}: High frequency band power derived from R-R intervals; significance for p<0.05

Table 20. Comparison of the baroreflex sensitivity (sequence method) data of the fainter and non-fainter groups

	Basal			0-5 min ('Phase 1')			5-10 min ('Phase 2')		
	Fainters (n=188)	Non-fainters (n=219)	P	Fainters	Non-fainters	P	Fainters	Non-fainters	P
BEI (%)	72±30.2	65±25.7	0.0119	54.6±23.1	62.1±24.9	**0.0019**	58.3±26.8	67.2±23.2	**0.0004**
BRS (ms/mmHg)	9±4.7	8.5±5.1	0.373	5.7±3	8.2±2.4	**0.0001***	5.9±3.4	8.6±3.6	**0.0001**
	10-15 min ('Phase 3')			15-20 min ('Phase 4')			Tilt-down		
	Fainters	Non-fainters	P	Fainters	Non-fainters	P	Fainters	Non-fainters	P
BEI (%)	56.5±29.2	75.7±20.4	**0.0001**	45.9±18.3	72.6±30.5	**0.0001**	68±14.7	69.8±27.5	0.4218
BRS (ms/mmHg)	5.3±3.6	8.8±3.8	**0.0001**	4.8 ±3.4	8.9±2.8	**0.0001**	7.5±4.1	8.1±3.1	0.0941

Table 21. Comparison of the baroreflex sensitivity (sequence method) data of the healthy control and non-fainter groups

	Basal			0-5 min ('Phase 1')			5-10 min ('Phase 2')		
	Controls (n=30)	Non-fainters (n=219)	P	Controls	Non-fainters	P	Controls	Non-fainters	P
BEI (%)	62.1±25.9	65±25.7	0.5631	60.4±33.7	62.1±24.9	0.7381	65.7±23.6	67.2±23.2	0.7406
BRS (ms/mmHg)	8.3±5.9	8.5±5.1	0.8436	8.1±3.8	8.2±2.4	0.8438	8.5±4.9	8.6±3.6	0.8919

	10-15 min ('Phase 3')			15-20 min ('Phase 4')			Tilt-down		
	Controls	Non-fainters	P	Controls	Non-fainters	P	Controls	Non-fainters	P
BEI (%)	72,8±34.1	75.7±20.4	0.5075	73.1±28.3	72.6±30.5	0.9324	72.1±32.5	69.8±27.5	0.6749
BRS (ms/mmHg)	8.7±4.1	8.8±3.8	0.8936	9.1±2.4	8.9±2.8	0.7096	8.6±1.9	8.1±3.1	0.3903

CHAPTER 6

SUPPLEMENTARY MATERIAL

THE MANAGEMENT OF REFLEX SYNCOPE

Table 22. Evolution of haemodynamic and autonomic nervous system markers during tilt training (Session 1 vs Session 9; Basal and first 10 minutes)

	Basal			5 min			10 min		
	S1	S9	P	S1	S9	P	S1	S9	P
HR (bpm)	74,2±11,6	71,99±13,70	0.2121	95,7±16,2	75,4±14,5	**0.0001***	106,4±20,2	80,9±13,9	**0.0001***
SBP (mmHg)	115,4±14,3	114,72±11,43	0.7758	112,3±10,75	119,0±15,1	0.0503	107,9±14,4	121±16,4	**0.0006****
SV (ml)	62,1±35,4	69,12±17,80	**0.0026***	51,22±29,4	64,7±11,7	**0.0001***	49,5±28,1	64,9±11	**0.0001****
CO (l/min)	5,2±1,3	6,46±1,99	**0.0001***	4,8±1,2	6,2±2,0	**0.0001***	4,9±1,2	6,1±2,1	**0.0001****
TPR (dyne*s/cm^5)	1094,8±701,1	1426,30±354,01	**0.0001***	1091,4±830,7	1570,6±398,5	**0.0001***	1106,1±834,6	1579,2±392,1	**0.0001****
LF_{SBP} (mm Hg2)	11,7±6,6	16,3±2,0	**0.0001***	17,9±3,6	17,4±3,2	0.56	15,8±8,7	17,9±3,9	**0.030**
HF_{RRI} (ms2)	350,2±206,0	486,2±365,5	**0.0013***	310,4±304,9	464,6±218,5	**0.0001***	306,7±246,7	378,4±255,1	**0.0425***

S1: Session 1; S9: Session 9; HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; LF_{SBP}: Low-frequency band derived from SBP; HF_{RRI}: High-frequency band power derived from R-R intervals

Table 23. Evolution of haemodynamic and autonomic nervous system markers during tilt training (Session 1 vs Session 9; last 20 minutes)

	15 min			20 min			25 min			30 min		
	S1	S9	P	S1	S9	P	S1	S9	P	S1	S9	P
HR (bpm)	110,4±23,3	87,9±14,2	0.0001*	106,9±24,5	88,5±14,6	0.0001*	107,5±28,2	80,9±15,4	0.0001*	113,2±25,4	85,2±12,5	0.0001**
SBP (mmHg)	108,5±14,4	120,8±14,3	0.0010*	105,9±17,8	119,7±13,9	0.0007*	98,6±14,1	121,8±16,5	0.0001*	99,9±10,2	123,8±16,1	0.0015*
SV (ml)	50,56±29,6	64,9±11,4	0.0001*	49,7±31,3	64,4±10,6	0.0001*	47,9±31,2	64,4±10,6	0.0001*	53,4±36,2	72,1±13,6	0.0001**
CO (l/min)	4,8±1,7	6,2±2,1	0.0001*	4,5±1,6	6,3±2,1	0.0001*	4,9±1,2	6,6±2,0	0.0001*	4,8±1,3	6,8±2,0	0.0001**
TPR (dyne*s/cm^5)	1079,4±809,3	1575,2±403,4	0.0001*	1026,3±807,9	1564,8±331,5	0.0001*	951,5±788,7	1588,4±352,1	0.0001*	879,8±748,9	1350,4±334,0	0.0001**
LF_{SBP} (mm Hg2)	14,2±7,3	18,5±4,6	0.0001*	13,3±8,9	18,2±6,7	0.0001*	16,7±9,7	18,9±5,4	0.049*	10,3±4,3	17,8±3,9	0.0001**
HF_{RRI} (ms2)	290,3±202,7	460,1±350,2	0.0001*	306,3±252,2	360,2±333,1	0.1938	300,2±171,1	372,3±269,9	0.0238**	280.8±270,2	491,9±317,4	0.0001**

S1: Session 1; S9: Session 9; HR: Heart rate; SBP: systolic blood pressure; SV: Stroke volume; CO: cardiac output; TPR: Total peripheral resistance; LF_{SBP}: Low-frequency band derived from SBP; HF_{RRI}: High-frequency band power derived from R-R intervals

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REVIEW ARTICLE

Insights into the background of autonomic medicine

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KEYWORDS
Autonomic physiology;
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Autonomic evaluation;
Tilt test

Abstract Knowledge of the physiology underlying the autonomic nervous system is pivotal for understanding autonomic dysfunction in clinical practice. Autonomic dysfunction may result from primary modifications of the autonomic nervous system or be secondary to a wide range of diseases that cause severe morbidity and mortality. Together with a detailed history and physical examination, laboratory assessment of autonomic function is essential for the analysis of various clinical conditions and the establishment of effective, personalized and precise therapeutic schemes. This review summarizes the main aspects of autonomic medicine that constitute the background of cardiovascular autonomic dysfunction.

PALAVRAS-CHAVE
Fisiologia autonómica;
Regulação cardiovascular;
Reflexo barorrecetor;
Reflexo quimiorrecetor;
Avaliação autonómica;
Teste de *tilt*

Introdução à medicina autonómica

Resumo O conhecimento subjacente à fisiologia do sistema nervoso autónomo é fundamental para se entender a disfunção autonómica na prática clínica. A disfunção autonómica pode resultar primariamente de modificações do sistema ou, secundariamente, a uma série de patologias conducentes a morbilidade ou mortalidade. Juntamente com a colheita detalhada da história clínica e do exame objetivo, a avaliação autonómica laboratorial torna-se essencial na análise de algumas condições clínicas e no estabelecimento de esquemas terapêuticos mais eficazes, refinados e personalizados. Assim, nesta revisão sumarizam-se os aspetos mais relevantes da fisiologia autonómica subjacente a disfunção autonómica cardiovascular.

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Introduction

Attempts to bridge the gap between basic and clinical science, known as the translational approach to medical knowledge, contribute to better clinical practice, enabling a comprehensive interpretation of pathophysiological mechanisms, more accurate diagnosis and establishing more effective treatment. Advances in autonomic research in recent years and the development of implantable devices that affect autonomic tone mean there is a growing need to understand the scientific basis of autonomic medicine, in order to improve management of autonomic dysfunction in cardiology.

This review aims to provide a basis for understanding autonomic failure in cardiovascular disease. The first section deals with the basic aspects of autonomic function, while the second covers the most important reflexes. The third section describes the most common methods of autonomic evaluation.

The autonomic nervous system

Almost all bodily functions are dependent on the autonomic nervous system (ANS), which exerts precise control over visceral functions (Figure 1). However, the mechanisms through which the ANS exerts this control are not well understood.

Although the ANS is able to hide its own dysfunction, disautonomy, also known as autonomic failure, can occur due to functional failure, a physical defect in the nervous network, and as a result of the aging process. In these conditions, the system becomes over-activated, the resulting allostatic overload being believed to contribute to various diseases, including hypertension, atrial fibrillation and other cardiac arrhythmias, ischemic heart disease, obesity, diabetes, atherosclerosis, sleep apnea, metabolic syndrome and congestive heart failure.[1–14]

The ANS is one of the two major divisions of the peripheral nervous system (the other being the somatic nervous system). The ANS functions mainly through negative feedback mechanisms and via reflex arcs, using specific neuronal pathways in the periphery and a specific central organization to perform precise and flexible actions. In the present text, we will use Langley's neuroanatomical terminology and the terms sympathetic, parasympathetic and enteric will only refer to the motor portion of the autonomic reflex arc (Figure 2). This arc also includes integrative centers located in the central nervous system (CNS) (the central autonomic network) to which sensory information is conveyed from peripheral sensors located in specific reflexogenic areas.[6,12,14,15]

Visceral afferent pathways

Afferent pathways are the interface between the visceral organs and the CNS. Most afferent fibers are unmyelinated, but myelinated fibers can also conduct autonomic sensory information.[15] There are two types of visceral afferents, primary afferent fibers and enteric afferent fibers. The latter respond to chemical and mechanical events and their cell bodies are located in the gastrointestinal tract walls.[15,16] Primary afferent inputs are carried orthodromically to the spinal cord, brain stem or prevertebral sympathetic ganglia. The degree to which these afferent neurons are physiologically specific is determined by the responses evoked by chemical or mechanical stimulation.[15–17] Most of these afferents transmit information from the viscera to the CNS,[4,18–20] but some also make contact with sympathetic preganglionic neurons in the prevertebral ganglia.[21] These anatomical relations indicate that in addition to their central actions, visceral primary afferents (and also enteric afferent fibers) may also play a role in peripheral regulatory reflexes, mainly those that are active in pathological conditions through positive feedback mechanisms.[21]

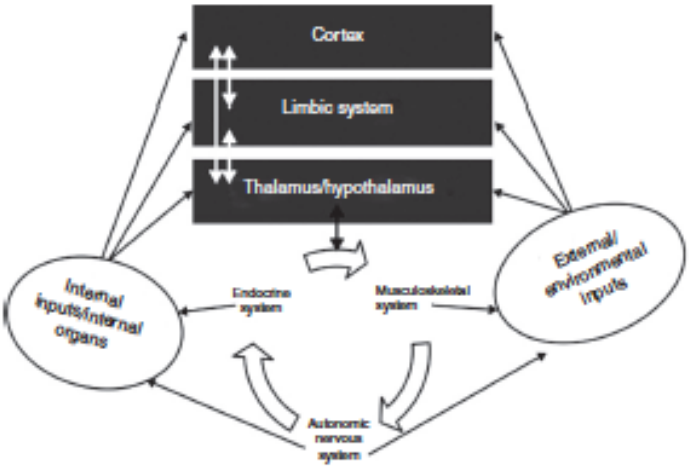

Figure 1 The interactions between the autonomic nervous system, the brain, the body and the environment. Adapted from Jänig.[16]

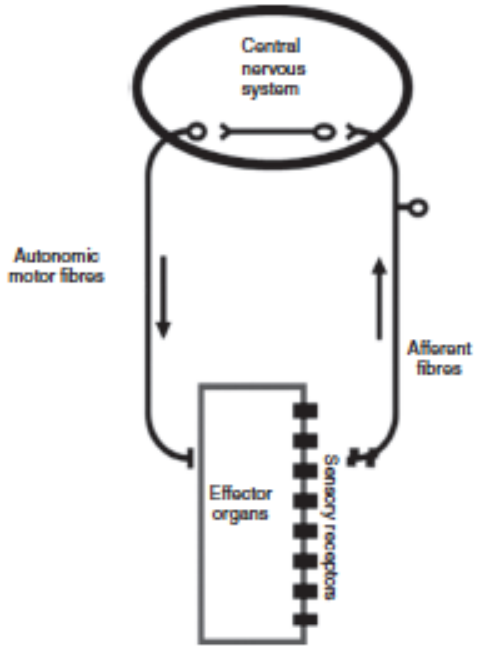

Figure 2 The autonomic reflex arc. The morphological relations between its different components are shown. The autonomic motor fibers include sympathetic, parasympathetic and enteric fibers. Adapted from Rocha.[106]

Afferent neurons are involved in two main functions: regulation of visceral actions, including organ-protective reflexes; and the transport of pain information, including pain from deep somatic tissues and regulation of hyperalgesia, deep pain and inflammation.[15,16,22] This dual function makes them fundamentally different from somatic afferents, in which sensory and regulatory properties cannot be separated; afferent impulses from skin or muscle trigger reflexes and behavioral regulation simultaneously with sensory experience, which is not the case with visceral afferents, as some stimuli from the latter never reach the level of consciousness, such as changes in blood pressure (BP) or gut distension.[15–17,23] The majority of the viscera show dual afferent innervation, with most afferent fibers traveling in mixed parasympathetic nerves, such as the vagus and pelvic nerves.[24] The physiological significance of this dual innervation – afferent fibers being carried in sympathetic and parasympathetic nerves – is not fully understood, but data suggest that reflex and regulatory functions evoked by visceral stimulation are mainly triggered by activity in afferent fibers in the vagus and pelvic nerves, while visceral sensation, and in particular visceral pain, together with some visceral reflexes originating in the mesenterium, are mediated by afferent fibers in sympathetic nerves.[15,16]

Efferent pathways

Efferent pathways of the peripheral ANS have three major subdivisions: sympathetic, parasympathetic, and enteric. These systems are the building blocks of the motor part of the autonomic reflex arc and establish the final autonomic pathway,[25] as each of them consists of a series of pre- and postganglionic neurons that are synaptically connected in autonomic ganglia, which function as the connection between the brain centers and the target organ.

Each autonomic nerve pathway extending from the CNS to an innervated organ is a two-neuron chain (except to the adrenal medulla that in effect functions as a sympathetic ganglion). The cell body of the first neuron, located in the CNS, synapses with a second-order neuron, the cell body of which lies within an autonomic ganglion.[26] It is generally accepted that, except for the enteric nervous system, the organization of parasympathetic nervous pathways is simpler than that of the sympathetic nervous system. However, while this may be true for some pathways and target organs such as the pupillae and ciliary muscles, it seems unlikely to be the case for other target organs like the heart or the urinary bladder.[13,27–29]

Sympathetic efferent pathways

Sympathetic preganglionic neurons are a heterogeneous population. Morphologically, they vary in somal shape, size and dendritic arborization, giving rise to either non-myelinated or myelinated axons, which are not selective in relation to a single target. In the spinal cord, sympathetic preganglionic neurons are located in four nuclei: the lateral funicular, intermediolateral, intercalated, and central autonomic nuclei, of which the most important for cardiovascular regulation is the intermediolateral nucleus.

Independently of how preganglionic sympathetic neurons are positioned within the different spinal nuclei, they are segmentally organized, this arrangement providing the anatomical substrate for a more general rostrocaudal functional topography.[15,30]

Sympathetic preganglionic neurons exhibit a low level of tonic activity. This may reflect the influence of both intrinsic membrane properties and the integration of excitatory and inhibitory postsynaptic potentials. Their activity is regulated by segmental inputs from visceral and somatic afferents and supra-spinal pathways.[31] According to their biophysical properties and functional properties, sympathetic preganglionic neurons can be divided into phasic (rapidly adapting), tonic (slowly adapting), and those with a long after-depolarization.

Parasympathetic efferent pathways

Compared to the quantity of research on sympathetic reflex responses, there have been relatively few studies on the parasympathetic system. There are various reasons for this but they are all due to the fact that most parasympathetic ganglia are located close to or within the target organ walls, and therefore the postganglionic parasympathetic neuron is very short. These less easily morphologically-defined neuronal structures, together with less pronounced target organ parasympathetic innervation, hamper neural recording and peripheral modulation of parasympathetic circuits.[32,33]

In neuroanatomical terms, brain stem parasympathetic preganglionic nuclei include the Edinger-Westphal nucleus, the superior and inferior salivatory nuclei, the dorsal motor vagal nucleus and the nucleus ambiguus. Neurons of the

ventrolateral nucleus ambiguus provide the main parasympathetic innervation of the cardiac ganglia, which innervate the heart, esophagus, and respiratory airways.[34]

The heart is innervated by at least two parasympathetic pathways. One, which acts directly on the sinus node and other pacemaker cells, is involved in heartbeat regulation and atrial inotropism. These myelinated neurons emerge from the nucleus ambiguus and are activated by baroreceptor stimulation. They can show spontaneous rhythmic activity, the absence of activity coinciding with inspiration and activation being simultaneous with expiration. This coupling to central respiratory activity is the basis of respiratory sinus arrhythmia. The second pathway is formed mainly of unmyelinated neurons that originate in the dorsal motor nucleus of the vagus. Some of these neurons can also show spontaneous activity that is not modulated by the central respiratory drive or by baroreceptor activity; in the heart, their main function appears to be to induce coronary vasodilation when activated.[35–38]

Dual autonomic innervation

The two divisions of the ANS rarely operate independently, and autonomic responses generally represent the regulated interplay of both divisions (Table 1). The heart, glands and smooth muscles are innervated by both sympathetic and parasympathetic fibers (dual innervation). Moreover, they are usually activated reciprocally, i.e. when the activity of one division increases, the activity of the other decreases. Dual innervation by nerve fibers that cause opposite responses provides a fine degree of control over the effector organ. The sympathetic system promotes responses that prepare the body for strenuous physical activity in emergency or stressful situations, the sympathetic response being characterized by tachycardia, hypertension and increased blood flow to the skeletal muscles, heart, and brain, release of glucose by the liver and dilatation of the pupils.[26,30] The parasympathetic system dominates in quiet, relaxed situations; under nonthreatening circumstances, the body can perform its own general housekeeping activities.[26]

There are several exceptions to the general rule of dual reciprocal innervation by the two branches of the ANS. Innervated blood vessels (most arterioles and veins) receive only sympathetic nerve fibers. Regulation is accomplished by increasing or decreasing the firing rate above or below the tonic level in these sympathetic fibers. The only blood vessels to receive both sympathetic and parasympathetic fibers are those supplying the penis and clitoris. Most sweat glands are innervated only by sympathetic nerves. Salivary glands are innervated by both autonomic divisions, but sympathetic and parasympathetic activity are not antagonistic; both stimulate salivary secretion, but saliva volume and composition differ depending on which autonomic branch is dominant.[26]

Central autonomic network

Central autonomic pathways are organized at two levels, some for reflex adjustments of the end organ, while others are organized in a more complex fashion by connecting to higher neural centers, forming a central autonomic circuit capable of producing wide-ranging autonomic, endocrine, and behavioral responses (Figure 3).[15,31]

Central control of autonomic function involves several interconnected areas distributed throughout the neuraxis.[39] This central autonomic network has a critical role in moment-to-moment control of visceral function, homeostasis, and adaptation to internal or external challenges.[14,15,31,39]

The network functions on four closely interconnected hierarchical levels: spinal, bulbopontine, pontomesencephalic and forebrain (Figure 4). Of these, the bulbopontine level is involved in reflex control of circulation and respiration, and is the location of the nucleus tractus solitarii (NTS), the first relay station for reception of peripheral visceral information, and the rostroventrolateral medulla (RVLM), which contains bulbospinal neurons that are fundamental for the control of vasomotor, cardiac and respiratory function and for the coordination of various cardiovascular reflexes. These RVLM neurons also control hypothalamic function and neurons of the ventral respiratory group involved in respiratory rhythmogenesis.[14,15,31,39] Located more rostrally, the parabrachial nucleus is a major relay center for the convergence of various types of sensory information (visceral, nociceptive and thermoreceptive), and contains separate subnuclei linked to gastrointestinal, cardiovascular and respiratory regulation together with clusters of neurons involved in osmo- and thermoregulation.[14,15,31,39] The midbrain periaqueductal gray integrates autonomic, somatic and antinociceptive responses to stressful stimuli. Morphologically, it is divided into columns, which control cardiorespiratory and urinary function as well as pain, thermoregulation and reproductive function.[14,15,31,39]

The forebrain level includes the hypothalamus and components of the anterior limbic circuit, including the insular cortex, anterior cingulate cortex and amygdala. Playing a central role in neuroendocrine integration critical for homeostasis and integrative adaptive responses, the hypothalamus and periaqueductal gray are also involved in the defense reaction, an acute but active adaptation to stressful stimuli leading to sympathetic activation and tachycardia, hypertension, positive inotropism, increased stroke volume and cardiac output, redistribution of blood flow, tachypnea, baroreflex inhibition and facilitation of the chemoreceptor reflex.[40] In this reaction, the paraventricular hypothalamic nucleus coordinates neuroendocrine integration, including sympathoexcitation, secretion of vasopressin (VP) and activation of the adrenomedullary and adrenocortical systems.

Autonomic cardiovascular reflexes

Cardiovascular autonomic reflexes include short-term, moment-to-moment, mechanisms that regulate heart rate (HR) and BP. They result from activation of peripheral receptors whose afferents link to the CNS via the glossopharyngeal and vagus nerves.[5] CNS processing of afferent information is followed by regulation of autonomic efferent pathways and adjustment of cardiovascular parameters.[41] Central BP control involves both sympathetic and parasympathetic nervous systems. The sympathetic system increases stroke volume, HR and total peripheral resistance,

Table 1 Some effects of autonomic nervous system activity. Adapted from Vander et al.[30]

Effector organ	Receptor type	Sympathetic effect	Parasympathetic effect
Eyes			
Iris muscle	Alpha	Contracts radial muscle (widens pupil)	Contracts sphincter muscle (makes pupil smaller)
Ciliary muscle	Beta	Relaxes (flattens lens for far vision)	Contracts (allows lens to become more convex for near vision)
Heart			
Sinoatrial node	Beta	Increases heart rate	Decreases heart rate
Atria	Beta	Increases contractility	Decreases contractility
Atrioventricular node	Beta	Increases conduction velocity	Decreases conduction velocity
Ventricles	Beta	Increases contractility	Decreases contractility slightly
Arterioles			
Coronary	Alpha	Constricts	–
	Beta	Dilates	
Skin	Alpha	Constricts	–
Skeletal muscle	Alpha	Constricts	–
	Beta	Dilates	
Abdominal viscera	Alpha	Constricts	
	Beta	Dilates	–
Salivary glands	Alpha	Constricts	Dilates
Veins	Alpha	Constricts	–
	Beta	Dilates	
Lungs			
Bronchial muscle	Beta	Relaxes	Contracts
Bronchial glands	Alpha	Inhibits secretion	Stimulates secretion
	Beta	Stimulates secretion	
Salivary glands	Alpha	Stimulates watery secretion	Stimulates watery secretion
	Beta	Stimulates enzyme secretion	
Stomach			
Motility, tone	Alpha and beta	Decreases	Increases
Sphincters	Alpha	Contracts	Relaxes
Secretion		Inhibits (?)	Stimulates
Intestine			
Motility	Alpha and beta	Decreases	Increases
Sphincters	Alpha	Contracts (usually)	Relaxes (usually)
Secretion	Alpha	Inhibits	Stimulates
Gallbladder	Beta	Relaxes	Contracts
Liver	Alpha and beta	Glycogenolysis and gluconeogenesis	–
Pancreas			
Exocrine glands	Alpha	Inhibits secretion	Stimulates secretion
Endocrine glands	Alpha	Inhibits secretion	–
	Beta	Stimulates secretion	
Fat cells	Alpha and beta	Increases fat breakdown	–
Kidneys	Beta	Increases rennin secretion	–
Urinary bladder			
Bladder wall	Beta	Relaxes	Contracts
Sphincter	Alpha	Contracts	Relaxes
Uterus	Alpha	Contracts in pregnancy	Variable
	Beta	Relaxes	
Reproductive tract (male)	Alpha	Ejaculation	Erection
Skin			
Muscles causing hair erection	Alpha	Contracts	–
Sweat glands	Alpha	Localized secretion	Generalized secretion
Lachrymal glands	Alpha	Secretion	Secretion

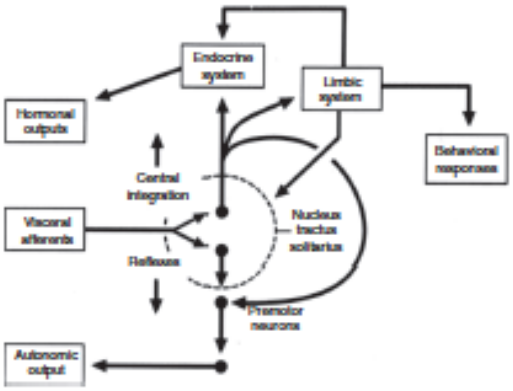

Figure 3 Diagram of the two main types of visceral information processing by the central autonomic network. Information originating in the periphery is processed and produces either a reflex response or integrated autonomic, hormonal and behavioral output, the classic example of which is thermoregulation in the hypothalamus. Adapted from Loewy and Spyer.[15]

increasing BP, while parasympathetic activation decreases HR, cardiac inotropism and stroke volume, reducing BP.[42] Several receptor types are involved in the modulation of sympathetic and parasympathetic activity, including arterial baroreceptors, cardiopulmonary receptors and arterial chemoreceptors.[6,43–48]

Baroreceptor reflex

The baroreceptor reflex is the major mechanism for adjusting BP. It is initiated by stimulation of arterial baroreceptors, which are nerve endings found in vascular and cardiac reflexogenic areas that are sensitive to stretching during the cardiac cycle. In blood vessels, the most important baroreceptors are located in the carotid sinus, aortic arch and mesenteric circulation.[49] The aortic arch receptors and medullar cardiovascular centers communicate through the vagus nerve, while it is Hering's nerve, a branch of the glossopharyngeal nerve, that conveys information from the carotid sinus receptors.[50] Arterial baroreceptors play a key role in short-term adjustments of BP, maintaining it within a normal range by acting on cardiac output, peripheral resistance and inotropism.[51,52]

Baroreceptors respond to the distension and deformation that local BP changes elicited by phases of the cardiac cycle induce in the vessel and that change the frequency of nerve impulses that are carried to the NTS.[53,54] Changes in baroreceptor activity also affect breathing. As an example, in vivo studies on anesthetized vagotomized dogs showed that the carotid body chemoreceptor reflex response was eliminated by surgically excluding the carotid bodies from the carotid sinus baroreceptor area.[55]

Baroreceptors are also involved in secretion of VP,[56] particularly in response to hypotension, possibly due to neuronal projections from the NTS to the hypothalamus.[57] When the baroreceptor reflex is activated by a reduction in BP, an increase in VP secretion is observed,[58,59] and intact arterial baroreceptors are essential to maintain BP and VP secretion.[60,61] However, in this situation, their action is reinforced by facilitation of the chemoreceptor reflex in the NTS, due to the nature of the stimulus.[60]

Several studies have examined the role of the baroreceptor reflex in the long-term regulation of sympathetic activity, as central resetting of the baroreceptor-sympathetic reflex may be an important component of the mechanism causing sustained changes in renal sympathetic activity. However, little is known about the mechanisms that could cause such resetting.[62]

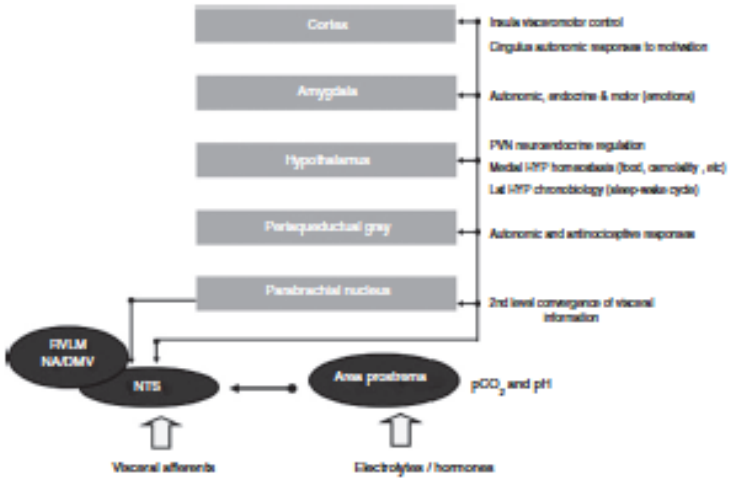

Figure 4 Central autonomic control areas and levels of interaction of autonomic control. DMV: dorsal motor nucleus of the vagus; HYP: hypothalamus; Lat HYP: lateral hypothalamus; NA: nucleus ambiguous; NTS: nucleus tractus solitarii; PVN: paraventricular nucleus of hypothalamus; RVLM: rostral ventrolateral medulla.

Chemoreceptor reflex

Arterial chemoreceptors are highly specialized cells that can detect changes in the partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2) and pH in the blood. Peripheral chemoreceptors are more sensitive to changes in pO_2 than in pCO_2 or pH, while central chemoreceptors respond primarily to changes in pCO_2 and pH.[62]

Peripheral chemoreceptors are mainly located in the aortic and carotid bodies, but may also be found in the mesenteric circulation. The carotid bodies are located bilaterally at the bifurcation of the common carotid artery, while aortic bodies are located between the pulmonary artery and the aortic arch.[50] Carotid bodies are more sensitive to hypoxia and hypercapnia, and detect changes in blood gas tension, while aortic baroreceptors are more sensitive to anemia, carboxyhemoglobinemia and systemic hypotension.[63] Thus, carotid bodies monitor the ventilation/perfusion ratio and aortic bodies are responsible for reflex control of systemic vascular resistance. Central chemoreception was initially localized to areas on the ventral medullary surface in the area prostrema, but there is substantial evidence that many sites participate in central chemoreception, some located at a distance from the ventral medulla.[64]

Changes in the partial pressure of gases or pH detected by peripheral chemoreceptors are sent to the NTS through the vagus or the glossopharyngeal nerve.[65] Stimulation of chemoreceptors increases the activity of NTS cells. These cells simultaneously excite neurons in the vagal nuclei and RVLM, leading to an increase in sympathetic and parasympathetic tone, which results in ventilatory adjustments – increased air flow volume, respiratory rate and breathing volume – that play an important role in the reflex control of ventilation.[66] In addition to ventilatory responses, chemostimulation also induces changes in the cardiovascular system such as tachycardia and vasoconstriction that maintain the chemical composition of the blood and tissue perfusion at optimal levels.

It has been suggested that peripheral chemoreceptors have a tonic excitatory influence on cardiovascular control due to sympathetic activation, thus contributing to maintenance of BP levels.[67]

Cardiac reflexes

There are also volume receptors located in the right atrium and venae cavae that respond to blood volume decreases by reducing their firing rates. The afferents of these receptors join the vagus nerve and terminate in the NTS, synapsing with neurons projecting to the PVN.[68] This pathway is activated by changes in blood volume of as little as 8-10%.[69,70] Overall, activation of atrial receptors induces inhibition of sympathetic vasomotor tone and an increase in VP secretion, affecting renal function.

Other cardiac reflexes that regulate BP are the Bainbridge and Bezold-Jarisch reflexes. In the Bainbridge reflex, BP is indirectly regulated through HR changes. When right atrial volume increases, low-pressure stretch receptors initiate a reflex that increases HR through sympathetic nerve activation.[63] The Bainbridge reflex is not always active, its effect depending on HR, being stronger at lower than at higher HR values. In this way, the Bainbridge reflex acts in opposition to the baroreceptor reflex, which increases HR when stretching is decreased in hypotension or hypovolemia.[71] The Bezold-Jarisch reflex is a cardiac reflex that is sensitive to chemicals, evoking a strong cardiovascular depressor response leading to bradycardia and hypotension as a direct consequence of chemical stimulation of receptors in the ventricles or coronary circulation. The fall in BP is due to both bradycardia and vasodilation caused by inhibition of sympathetic vasomotor activity, and is also modulated by renin release and VP secretion.[72] Conversely, decreases in the activity of these inhibitory sensory receptors increase sympathetic activity, vascular resistance, plasma renin activity and VP secretion.[72]

Table 2 Summary of the provocative maneuvers for autonomic evaluation most commonly used in clinical practice.

Cardiovascular	Mixed maneuvers: head-up tilt (60°/70°); active standing; Valsalva maneuver
	Addressing mainly sympathetic branch: handgrip (isometric contraction), cold pressor test, mental arithmetic test
	Addressing mainly parasympathetic branch: deep breathing
	Liquid meal challenge
	Carotid sinus massage
Biochemical	Plasma noradrenaline, urinary catecholamines; plasma renin activity and aldosterone
Pharmacological	Noradrenaline–α-adrenoceptors, isoprenaline–β-adrenoceptors, atropine
Sudomotor	Thermoregulatory sweat test for central regulation
	Quantitative sudomotor axon reflex test for quantitative local sweat evaluation
	Silicone imprint with atropine iontophoresis for semi-quantitative local sweat evaluation
Eye	Schirmer's test
	Pupillary function tests
	Intraocular pressure tests

Evaluation of the autonomic nervous system

There are various standard provocative autonomic maneuvers designed to test the ANS with stimuli ranging from suprathreshold to maximum intensity, in order to observe evoked responses in target organs in terms of presence or absence, duration and magnitude (Table 2 and Figure 5). These maneuvers should be performed in a dedicated autonomic laboratory, which should have controlled temperature and humidity (20-23 °C and 25-35%, respectively) and an area of ~20 m^2. Depending on the type of assessment, each patient may undergo two or more different tests. All tests

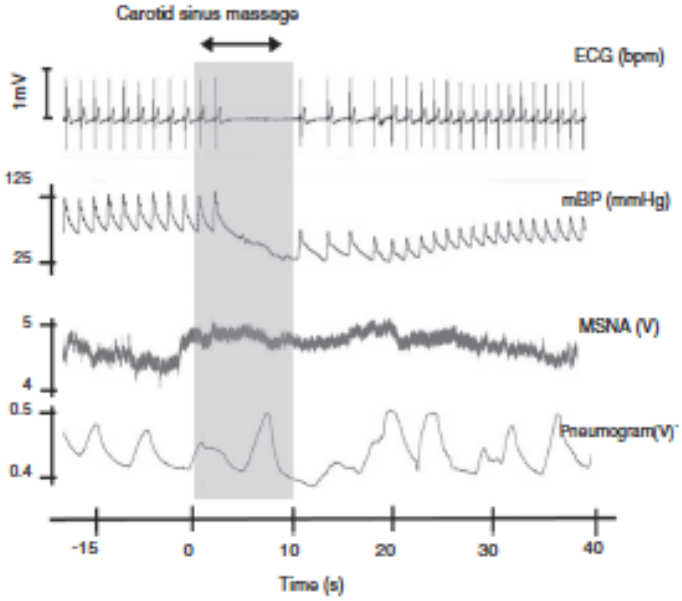

Figure 5 Carotid sinus massage. This figure shows the relation between cardiovascular variables, ventilation and muscle sympathetic nerve activity on sinus massage in a healthy subject. ECG: electrocardiogram; mBP: mean blood pressure; MSNA: muscle sympathetic nerve activity. Rocha and Laranjo, unpublished data.

should be performed under medical supervision by experienced technicians, whose training is critical to the success of any autonomic test battery.[11,14] These technicians must be familiar with sudomotor function, electrocardiography, beat-to-beat BP, blood flow recordings and computers, and must be able to identify and solve technical problems and recognize the main electrocardiographic abnormalities, as well as be knowledgeable in electrical safety and be trained in cardiopulmonary resuscitation.[11,14]

Patients undergoing autonomic evaluation should not consume food or tobacco at least four hours, and alcohol at least 12 hours, before the test, which should be performed during the morning, and should wear light clothing. Medications, particularly those that directly affect the ANS, should be discontinued according to the drugs' half-life and the patient's condition. In view of the considerable intra- and inter-individual variability in data, normal values are set by each laboratory and should be grouped by gender, age and decade of life. There are different ways of categorizing autonomic tests that take into account the target system, the type of variables recorded and the degree of invasion. Usually, due to the nature of the recording devices, most maneuvers target the cardiovascular system and are non-invasive in nature.

Autonomic maneuvers and the Ewing test battery

The evaluation and data analysis protocols should be appropriate to the study. A standard and the most common evaluation protocol is the Ewing test battery,[14] which assesses HR response to deep metronomic breathing, BP response to sustained handgrip and BP and HR response to the Valsalva maneuver and active standing.[11,14,73,74] Other non-invasive maneuvers, including the cold pressor test, mental stress test and tilt table, are also used in autonomic evaluation.

In the Valsalva maneuver, which assesses the sympathetic and parasympathetic reaction to baroreflex activation, the subject maintains an expiratory pressure of 40 mmHg/15 s with an open glottis. The test response is divided into four phases, two of which are reflex in nature (II and IV) and two mechanical (I and III). The results depend on the subject's position, age and gender, as well as the duration and intensity of expiratory pressure. In patients with autonomic dysfunction, there is typically a loss of both BP overshoot and reflex bradycardia (Figure 6).

The cold pressor test assesses sympathetic activation mediated by nociceptors, which is observed mainly through BP changes when the hand is immersed up to the wrist in ice-cold water at 4°C. This test, which is predominantly sympathetic, differs from the cold face test, in which the application of a cold stimulus to the face stimulates the trigeminal nerve and elicits bradycardia, and is related to the diving reflex. The cold pressor test, the mental stress test and the handgrip test are the provocative maneuvers mainly used for sympathetic evaluation.

On deep metronomic breathing, autonomic function is assessed with the patient breathing metronomically at a rate of six cycles/min for three minutes, which maximizes respiratory sinus arrhythmia (Figure 7). Changes in HR with deep breathing are a parameter of parasympathetic cardiac

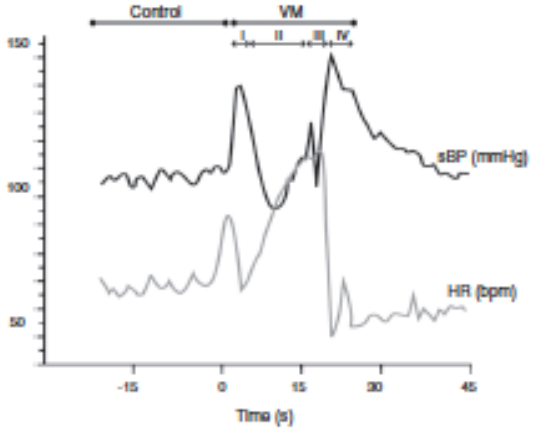

Figure 6 The Valsalva maneuver. Data from a normal subject, in which the Valsalva maneuver has four phases: (I) there is a brief decrease in heart rate (HR) and increase in systolic blood pressure (BP) due to aortic compression; (II) BP first decreases and then increases; (III) in this phase, due to the release of strain, consecutive declines in BP and increases in HR are observed, preceding a BP overshoot due to persistent sympathetic activity, together with normalization of venous return (IV). The increased BP mediates baroreflex-induced bradycardia and is quantified using the Valsalva ratio, the ratio of the highest HR (II) to the lowest HR (IV). Results depend on the position, age and gender of the subject, as well as the duration and intensity of expiratory pressure. In patients with autonomic dysfunction, there is typically a loss of both BP overshoot and reflex bradycardia. VM: Valsalva maneuver. Adapted from Xavier et al.[74]

control.[11,14] The subject's position and body weight, rate and depth of breathing, hypocapnia, sympathetic activity, and use of salicylates and other drugs influence HR variability during deep breathing.

The Ewing battery uses the 30:15 ratio together with blood pressure evaluation in order to assess cardiovascular responses to an active orthostatic challenge. The 30:15 ratio is calculated as the shortest RR interval around the 15th beat divided by the longest RR interval around the 30th beat after standing. Simultaneously with changes in HR there is a physiological decrease in BP. However, if this fall in systolic BP is at least 20 mmHg or in diastolic BP at least 10 mmHg within 3 min of standing, the BP changes are defined as orthostatic hypotension, a sign of cardiovascular autonomic failure.

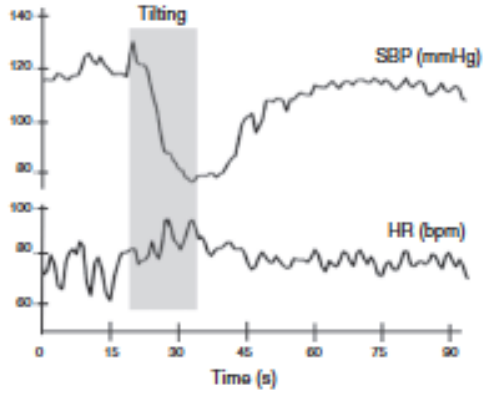

Figure 8 Normal heart rate and blood pressure responses to head-up tilt testing. In this test the subject lies on a tilt test table, which is then tilted upward at 60°–70° after a resting period of at least 5 min. Patients with different degrees of cardiovascular autonomic impairment may show delayed adaptation to the orthostatic challenge or may even be unable to adapt and have a syncopal event. HR: heart rate; SBP: systolic blood pressure. Adapted from Ducla-Soares et al.[73]

Autonomic evaluation of active standing can be complemented by the head-up tilt test. In theory, this detects the hemodynamic modifications elicited by baroreceptor reflex activation without the interference of the muscular pump of the legs. However, in practice this rarely occurs, as subjects usually develop an alerting reaction when they see the bed beginning to tilt, which is superimposed on the changes in BP and HR caused by baroreflex activation. The hemodynamic changes associated with head-up tilt testing are considered to consist of two stages: an initial acute cardiovascular response lasting around 30 s, and a stabilization phase consisting of an adaptation period 1-2 min after orthostasis followed by a late response to prolonged orthostasis lasting more than 5 min[11,14] (Figure 8).

Invasive and biochemical techniques applied to autonomic evaluation

Among the methods for measuring patients' sympathetic nervous system activity are tests that assess individual sympathetic nervous outflows, such as microneurography and measurement of norepinephrine (NE) spillover to plasma from the sympathetic nerves of individual

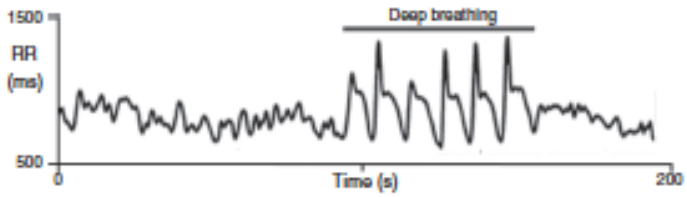

Figure 7 Heart rate response to deep breathing. A normal response showing the imprint of respiration in the heart rate recording during deep breathing. Adapted from Ducla-Soares et al.[73]

Table 3 Summary of time, frequency and modeling methods of baroreflex sensitivity assessment.

	Method	Brief description
Time domain	Sequences technique[108,109]	BRS as the mean of the slopes between SBP and RR values in each identified baroreflex sequence, considering SBP with a 1-beat lag
	Dual sequence method[110]	Equivalent to the sequences technique, identifying baroreflex sequences considering SBP and RR with a shift of up to 3 beats
	xBRS[111]	BRS as the slope between SBP and RR values over 10-s windows choosing the shift (up to 5 beats) that maximizes SBP and RR cross-correlation. SBP and RR series resampled at 1 Hz
	Events technique[112]	BRS as one global slope between SBP and RR values in all identified baroreflex events, considering SBP with a 1-beat lag with respect to RR
Frequency domain	Transfer function[113]	BRS as the mean magnitude of the transfer function between SBP and RR in the LF band
	Alpha technique[114]	BRS as the square root of the ratio between RR and SBP powers in the LF band
Model-based	Closed-loop bivariate[115]	Quantification of feedback and feedforward SBP and RR pathways, assuming a closed-loop SBP and RR system
	Closed-loop trivariate[116]	Quantification of feedback and feedforward SBP and RR pathways, considering two-way pathways between SBP, RR and respiration
	xAR[117]	Quantification of feedback and feedforward SBP and RR pathways considering respiration as an exogenous input in the SBP and RR loop and assuming a closed-loop SBP and RR system
	Causal analysis[118]	Quantification of BRS using an exogenous input model to divide RR variability into SBP-related and -unrelated parts

BRS: baroreflex sensitivity; LF: low-frequency; SBP: systolic blood pressure.

organs.[75,76] Alternatively, overall sympathetic activity can be assessed by analysis of plasma or urine catecholamine concentrations.[77,78]

Microneurography provides separate recordings of sympathetic nerve activity (SNA) traffic in muscle (MSNA) and skin (SSNA). MSNA reflects the vasoconstrictor signal to the skeletal muscle vasculature. It is highly sensitive to BP changes and is regulated by both arterial and cardiopulmonary reflexes. These reflexes do not affect SSNA. SSNA reflects vasomotor neural traffic to skin blood vessels with almost no sudomotor activity. The two recordings (MSNA and SSNA) differ significantly with regard to morphology. Studies have shown that measurement of sympathetic nerve activity from peripheral nerves is safe, accurate and reproducible. Furthermore, it has been shown that recordings from one limb can be reliably assumed to reflect recordings of sympathetic nerve activity to the muscle vascular bed throughout the body. The method's quantitative nature is also a significant advantage.[79,80]

Assessment of sympathetic activity based on plasma or urine NE concentration has significant limitations, as NE is subject to changeable reuptake dependent on the density of the basilar plexus and blood flow velocity in a specific organ. Moreover, circulating NE represents only a small fraction (5-10%) of the quantity of the neurotransmitter secreted from nerve terminals.[80] Measurement of plasma NE is, however, an improvement over assessment of urine epinephrine, NE and their precursors and metabolites, which was traditionally used to assess ANS tone.[79,80]

The NE spillover rate has advantages over the above-mentioned methods, since it assesses NE release from specific target organs. The NE radiolabeled method is based on intravenous infusion of small amounts of tritiated NE; tissue clearance of this substance is then subtracted from plasma NE values, and the remainder is a marker of spillover of the neurotransmitter from neuroeffector junctions. In steady-state conditions this spillover reflects the secretion of NE from sympathetic nerve terminals.[79,80] Invasive techniques measure total body and regional NE spillover in the heart, splanchnic and renal circulations, and the brain.[81]

Various methods are used for experimental quantification of sympathetic activity in animals.[75,82,83] Direct recordings of SNA (e.g. renal or lumbar) are commonly obtained in animals by the surgical implantation of recording electrodes into the appropriate sympathetic fibers.[84]

Evaluation of baroreflex function

Baroreceptor function is one of the most important mechanisms regulating moment-to-moment BP. It can be assessed by baroreflex sensitivity (BRS) tests that relate changes in heart period to a BP change. BRS can be assessed under dynamic or steady-state conditions, using physiological or pharmacological approaches. The most common methods include vasoactive drugs (Oxford method), the Valsalva maneuver, the neck chamber technique and analysis of spontaneous BP and HR fluctuations. The Oxford method uses phenylephrine (an alpha-1 adrenergic receptor agonist) to induce a rapid increase in BP (15-40 mmHg) together with HR changes. Modifications of the Oxford method assess BRS through sequential injections of depressor and pressor drugs. There is some controversy with phenylephrine concerning the selectivity of the reflex arc target, since other

reflex arcs, particularly the arterial chemoreceptor and the pulmonary mechano- and chemoreceptors, can also be activated. Applying negative or positive pressure to the neck selectively activates carotid baroreceptors and can act as an excitatory or inhibitory stimulus depending on whether positive or negative pressure is applied.

Computer-based techniques (Table 3) assess BRS by correlating spontaneous fluctuations of BP with consecutive HR changes. These computational methods can be divided into time domain, frequency domain and model-based approaches. Time (sequence) and spectral techniques have proven reliability and have become a standard tool in many autonomic testing devices. BRS can be determined by the sequential method.[85] This method searches ramps of BP and RR. A ramp defines a variation of at least 1 mmHg and 4 ms between adjacent values of BP and RR, respectively. This concept can only be applied to three or more cardiac cycles varying monotonically, either increasing or decreasing. When a BP ramp occurs at the same time as an RR ramp, a BRS event is identified. BRS can be determined by the mean BRS slope: ΔRRI(ms)/ ΔSBP(mmHg), where RRI is RR interval.[86] A steeper slope indicates high BRS, while a shallower slope indicates lower sensitivity. The baroreflex effectiveness index (BEI) is the ratio between the total number of BRS events and total number of pressure ramps, increasing or decreasing, for a given period. BEI is an indicator of the effectiveness of baroreceptor-mediated cardiac regulation.

Analysis of variability of biological signals

The fact that the rhythm of physiological signals is not entirely regular has raised the possibility of extracting an autonomic signature from these fluctuations using signal-processing techniques. Extremely complex neural mechanisms are responsible for these fluctuations, based mainly on interactions between the sympathetic and parasympathetic nervous system. They therefore represent a rich source of information that can provide considerable insight into the mechanisms of cardiovascular control.[87–93] Cardiovascular signals, in particular HR, are most commonly used. However, as in any biological assessment in which the environment affects the result, standardization is a problem, mainly because most autonomic evaluation is performed without a profound knowledge of methods or physiology, which can lead to confounding data and misinterpretation of physiological phenomena. Nonetheless, signal processing methods, when correctly used, are an important tool for identifying autonomic markers and for improving treatment and patient follow-up.

Signal processing can be applied in at least three domains – time, frequency and time-scale – which individually or together can identify different pathological response profiles, such as delays in adaptive responses to provocative maneuvers, dyssynergy between BP and HR responses, and/or exaggerated responses such as orthostatic hypotension, postural orthostatic tachycardia and syncope.[94]

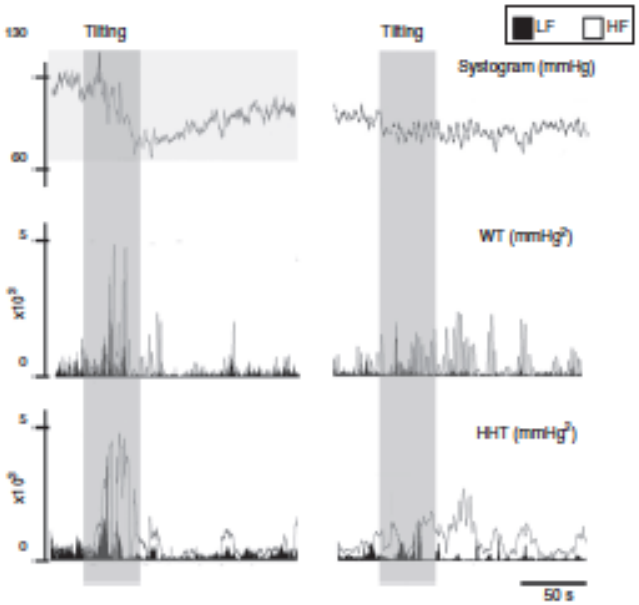

Figure 9 Signal processing of biological signals. Left: data from a normal subject; right: data from a patient with paroxysmal atrial fibrillation. The same signal, the systogram obtained from systolic blood pressure, has been treated with wavelets (Db12) and the Hilbert-Huang transform. The differences in the autonomic output of the two individuals can be clearly distinguished. Note that this example is merely illustrative, as the tachogram obtained from the RR interval for both patients is not shown. HF: high frequency; HHT: Hilbert-Huang transform; LF: low frequency; WT: wavelets.

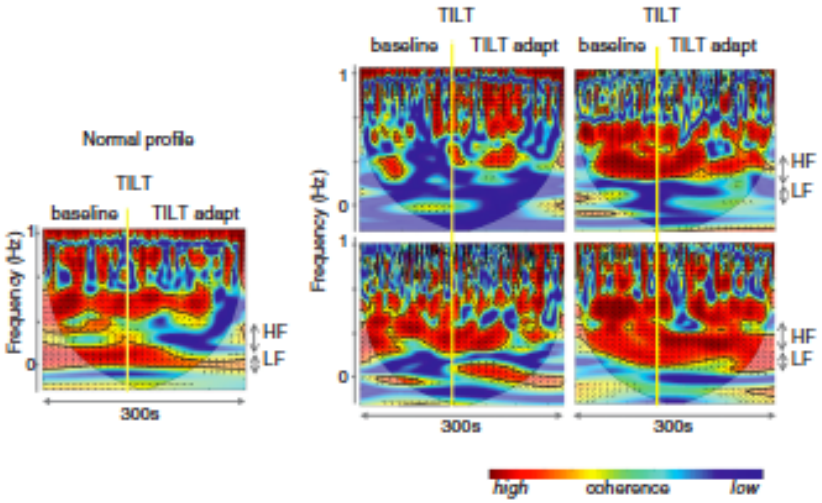

Figure 10 Autonomic analysis tools used to show reverse autonomic modulation in patients with reflex syncope. Left: changes in wavelet coherence evoked by a tilt maneuver in a normal subject. After tilting (vertical line) there is a fall in coherence, which reaches its minimum value approximately 20 s after tilting, recovering later to a significantly lower value than baseline; right: modification of coherence of heart rate and systolic blood pressure variability during a tilt training program designed to induce autonomic remodeling in patients with reflex syncope (A: baseline conditions before training program; B, C and D: 1st, 4th and 9th tilt-training sessions, respectively). The increase in coherence over the course of the training sessions, associated with increased baroreceptor remodeling, is reflected in improvement in band organization together with more intense orange/red color. These changes are more clearly seen after tilting (vertical line). Adapted from Laranjo et al.[107]

In particular, fast Fourier transform (FFT) and autoregressive spectral analysis applied to HR and BP signals have made an important contribution to autonomic evaluation.[87,94–97] FFT, using sinus functions of different frequencies and amplitudes, decomposes the signals to produce a power spectrum in which for human subjects three major frequency ranges can be recognized: very low frequency (VLF; <0.04 Hz), low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.4 Hz).[98] The VLF band is believed to be related to non-neural factors, such as temperature and hormones.[99] The HF band is dominated by the parasympathetic system,[98,100] while the LF band is believed to be mediated by both cardiac sympathetic and parasympathetic nervous outflows.

Guzzetti et al. reported that patients with essential hypertension are characterized by greater LF power and smaller HF power of RR interval variability during supine rest compared with normotensive subjects.[101] They also reported that the powers showed a smaller increase and decrease, respectively, during passive tilting. These observations were interpreted as indicating that cardiac sympathetic tone is increased and cardiac vagal tone and modulation are decreased in essential hypertension, a conclusion that is in agreement with previous studies in which autonomic cardiac modulation was investigated by different techniques.[102,103] FFT analysis, however, has important limitations, as it requires a stationary signal and a long period of data collection, of at least 5 min. Also, it cannot locate and follow changes in a frequency over time.

To overcome some of these limitations, like its application to nonstationary and nonlinear signals, a wavelet-based methodology has been proposed to determine the evolution over time of LF and HF frequencies.[73] Wavelet analysis is a linear non-stationary representation of signals in the time and frequency domains in which the original signal is decomposed in a shifted version of a basic function, called the mother wavelet, with a different base scale. A mother wavelet function is a non-periodic, oscillatory function that begins and ends at zero in the time domain.[104] However, although a good alternative to FFT, wavelets lack resolution, particularly at low frequencies (Figure 9). Wavelet coherence can also be used to analyze the degree of autonomic remodeling in patients under specific therapeutic schemes (Figure 10).

The Hilbert transform is a linear operator able to determine the instantaneous frequency of a signal, corresponding to the convolution of the input signal with the kernel. In order for amplitude, frequency and phase to have physiological application, the signal to be transformed must have an instantaneous null DC component.[105] Recently, Huang proposed fulfilling this condition through empirical mode decomposition (EMD), which can be applied to non-linear and non-stationary processes. The combination of the Hilbert transform with EMD results in what is known as the Hilbert-Huang transform.

Conclusion

The ANS has moved towards center stage in cardiovascular medicine. Dysregulation of this system contributes to cardiovascular disease, including hypertension, atrial fibrillation and other cardiac arrhythmias, ischemic heart

disease, obesity, diabetes, atherosclerosis, sleep apnea, metabolic syndrome and congestive heart failure, and is often associated with a more severe disease burden. However, there are serious gaps in our understanding of ANS function, and treatment options targeting the ANS are still in their infancy. It is important to begin by performing a thorough assessment of the ANS as it relates to the cardiovascular system. There are as yet no gold standard tests for autonomic testing and differences in test performances are an obstacle to comparing scientific and clinical data acquired in different laboratories. With this review, we hope to provide valuable help by focusing on standard tests for diagnosis of cardiovascular autonomic dysfunction.

Conflicts of interest

The authors have no conflicts of interest to declare.

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